ASSOCIATION OF ABNORMAL COAGULATION PROFILE AND LIVER ENZYMES WITH DENGUE INFECTION AND THEIR SIGNIFICANCE AS PREDICTORS OF ASSESSING SEVERITY OF DISEASE

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CERTIFICATE

This is to certify that the dissertation entitled **"ASSOCIATION OF ABNORMAL COAGULATION PROFILE AND LIVER ENZYMES WITH DENGUE INFECTION AND THEIR SIGNIFICANCE AS PREDICTORS OF ASSESSING SEVERITY OF DISEASE"** is the bonafide work of **Dr. VINOJ M** in partial fulfilment of the university regulations of the Tamil Nadu Dr.M.G.R Medical University, Chennai, for M.D General Medicine Branch I examination to be held in MARCH 2019.

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DECLARATION

I, **Dr.VINOJ M** solemnly declare that, this dissertation **"ASSOCIATION OF ABNORMAL COAGULATION PROFILE AND LIVER ENZYMES WITH DENGUE INFECTION AND THEIR SIGNIFICANCE AS PREDICTORS OF ASSESSING SEVERITY OF DISEASE"** is a bonafide record of work done by me at the Department of General Medicine, Govt. Rajaji Hospital, Madurai, under the guidance of **Dr.V. T. Premkumar, M.D**, Professor, Department of General Medicine, Madurai Medical College, Madurai.

This dissertation is submitted to The Tamil Nadu Dr. M.G.R Medical University, Chennai in partial fulfilment of the rules and regulations for the award of **M.D Degree General Medicine Branch-I;** examination to be held in MARCH 2019**.**

Place: Madurai

Date:

Dr.VINOJ M

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CONTENTS

INTRODUCTION

The dengue virus belongs to the genus Flavivirus, family Flaviviridae Aedea (Stegomyia) aegypti is the principal vector of dengue viruses**.**

Oneof the most severe arthropod borne viral diseases in terms of human mortality and morbidity is Dengue fever . The major cause of mortality is DHF/ DSS. Multiple reasons for abnormal haemostasis are vascular endothelial damage. Thrombopahty and coagulation abnormalities. Abnormalities in the coagulation and inflammation systems in dengue fever have been documented in various studies. The imbalance between coagulation and fibrinolysis serve as prognostic markers

Dengue virus infection spectrum spreads from an undifferentiated fever and dengue fever (DF) to dengue haemorrhagic fever (DHF) with shock and expanded dengue syndrome[1]. The hallmark of DHF is plasma leakage which may lead to shock. Both DF and DHF could have bleeding manifestations. Derangement of activated partial thromboplastin time (APTT) and the international normalised ratio (INR) is attributed to multiple factors.

This derangement in the coagulation system may be extrinsic or intrinsic which can be demonstrated by doing PT, and aPTT . Mortality can be reduced if these values can be determined and predicted to have been prolonged. These investigations aren’t costly too . Thus a study was done on the coagulation profile of patients admitted with dengue fever and correlation with severity was undertaken.

AIMS AND OBJECTIVES

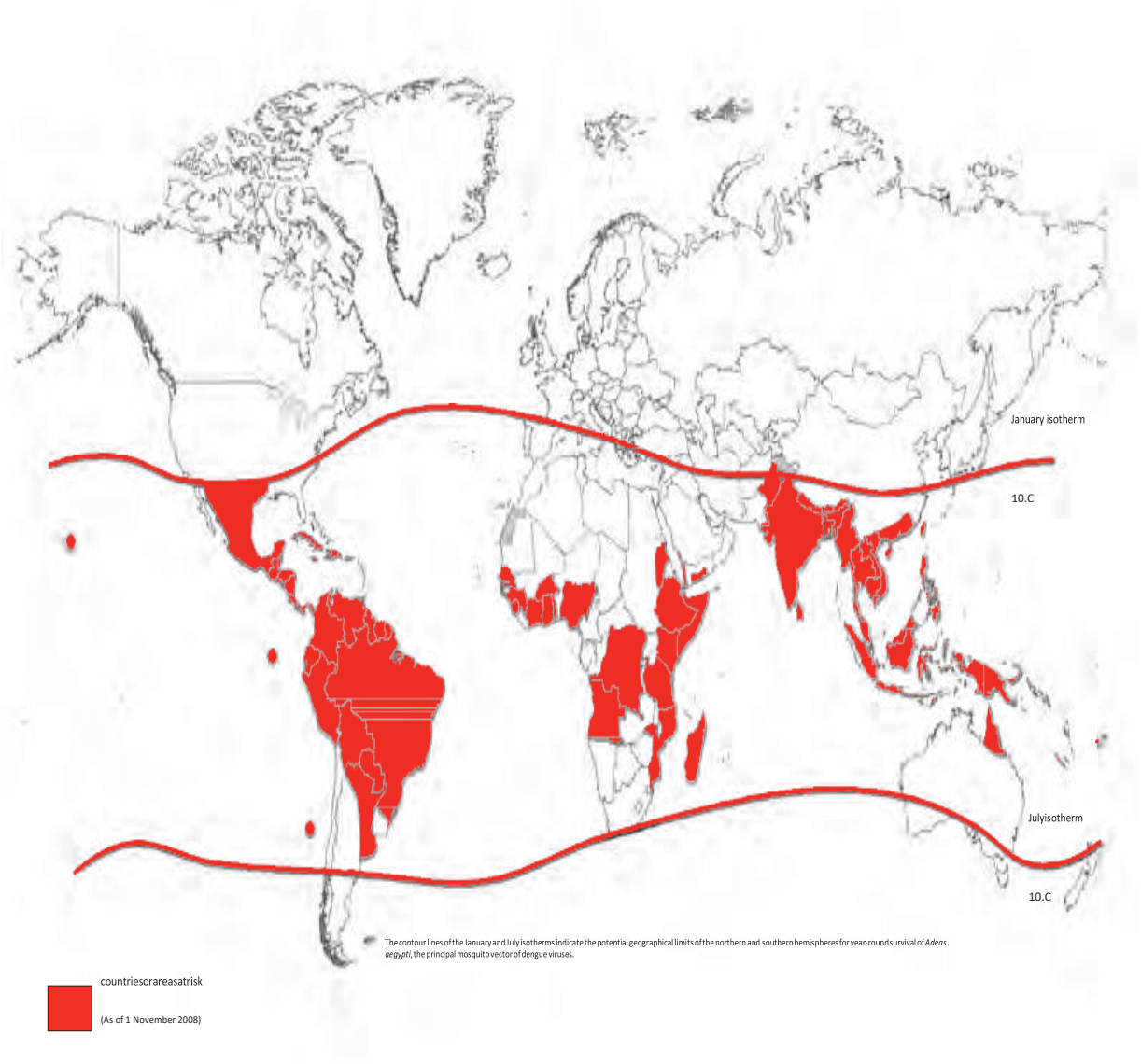
- To assess the liver enzyme and coagulation profile alterations among dengue patients .
- To follow up dengue patients over a period of 10 days and asses the progression of disease.
- To asses correlation between the abnormal lab parameters and their significance as early predictors of fluid leakage and bleeding

REVIEW OF LITERATURE

DENGUE EPIDEMIOLOGY

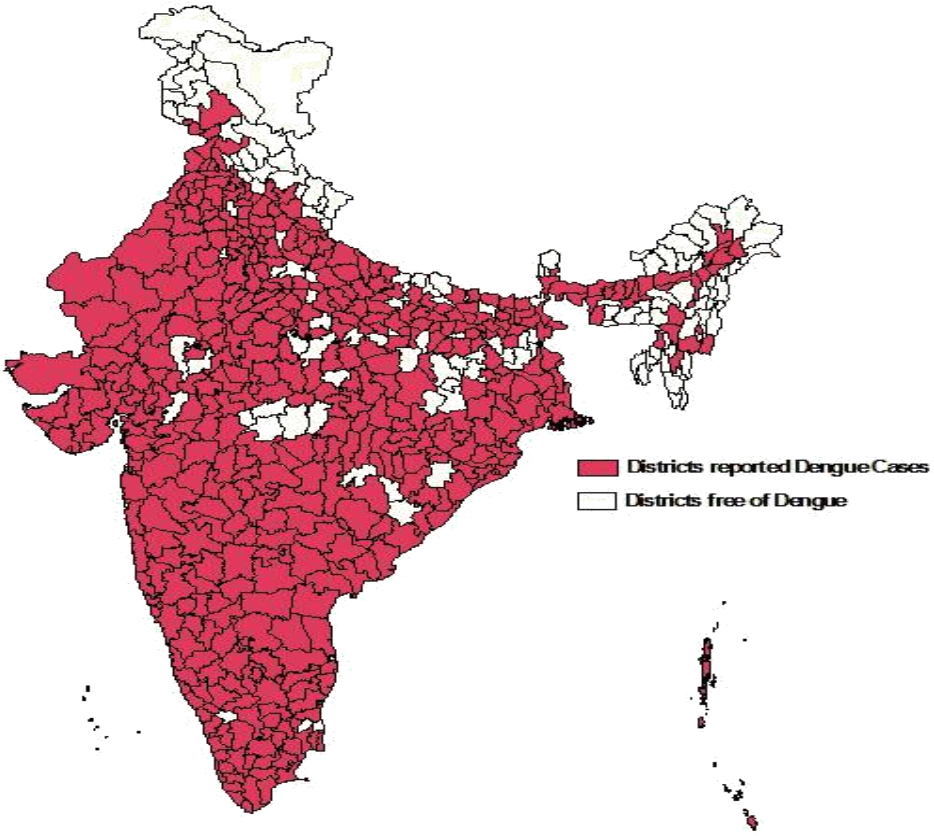
One of the most rapidly spreading mosquito-borne viral disease in the world in Dengue. In the last 50 years, incidence has increased 30-fold with increasing geographic expansion to new countries and, in the present decade, from urban to rural settings (Figure 1.1). An estimated 50 million dengue infections occur annually (Figure 1.2) and approximately 2.5 billion people live in dengue endemic countries (*1*). The 2002 World Health Assembly resolution WHA55.17 (*2*) urged greater commitment to dengue by WHO and its Member States. Of particular significance is the 2005 World Health Assembly resolution WHA58.3 on the revision of the International Health Regulations (IHR) (*3*), which includes dengue as an example of a disease that may constitute a public health emergency of international concern with implications for health security due to disruption and rapid epidemic spread beyond national borders.

Figure1.1Countries/areasatriskofdenguetransmission,2008



National scenario

Dengue virus was isolated in India for the first time in 1945. The first evidence of occurrence of dengue fever in the country was reported in 1956 from Vellore district in Tamil Nadu. Calcutta (West Bengal) saw the first dengue hemorrhagic fever (DHF) outbreak Of the 36 states/UTs, 35 (all except Lakshadweep) have reported dengue cases during the last two decades.



Recurring outbreaks of dengue fever(DF)/DHF have been reported from various states/ UTs—Andhra Pradesh, Chandigarh, Delhi, Goa, Haryana, Gujarat, Karnataka, Kerala, Maharashtra, Rajasthan, Uttar Pradesh, Puducherry, Punjab, Tamil Nadu and West Bengal.

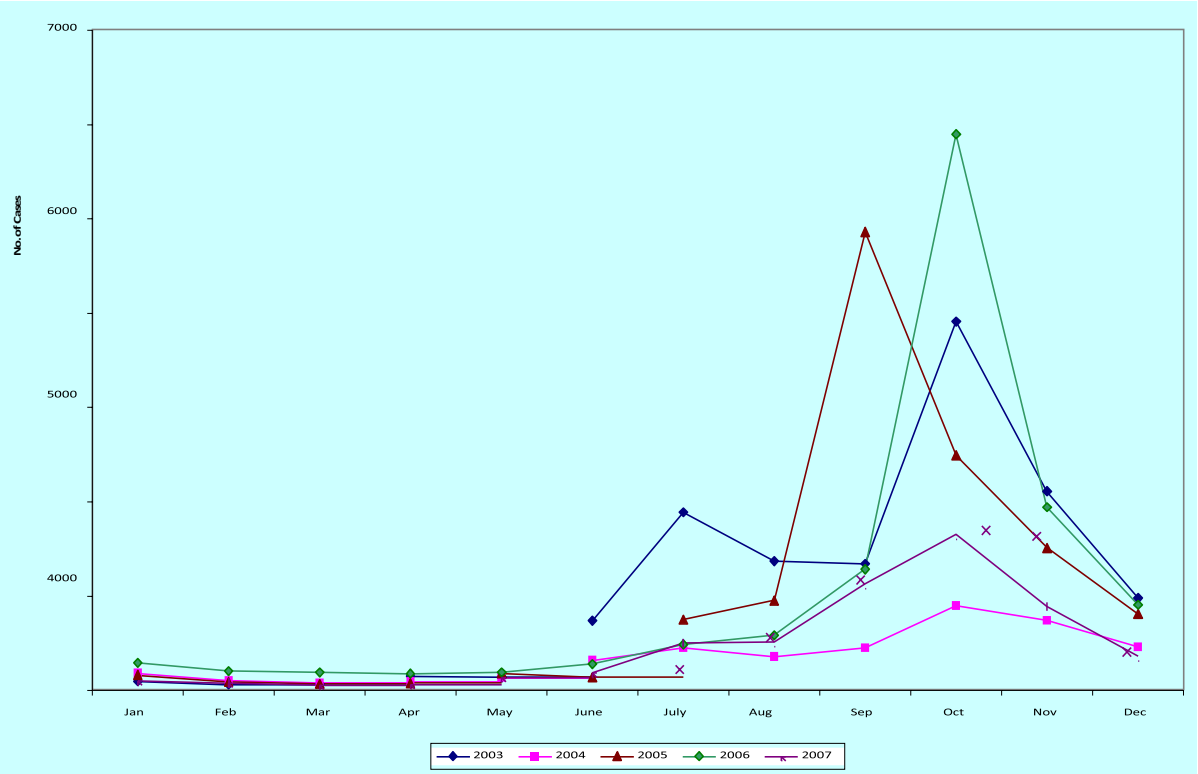
During 1996, one of the most severe outbreaks of DF/DHF occurred in Delhi, with 10 252 cases and 423 deaths being reported (country total being 16 517 cases and 545 deaths). In 2006, the country witnessed an outbreak of DF/DHF with 12 317 cases and 184 deaths. The incidence of dengue is increasing in the last few years. During 2010, a total of 28 292 cases were reported, which increased to 50 222 in 2012 and 75 808 in 2013 – the highest since 1991. The case fatality ratio (CFR – deaths per 100 cases) has declined from 3.3% in 1996 to 0.4% in 2010 after the national

guidelines on clinical management of DF/DHF/dengue shock syndrome (DSS) were developed and circulated in 2007. This further declined to 0.3% in 2013.[7,8]

Dengue virus

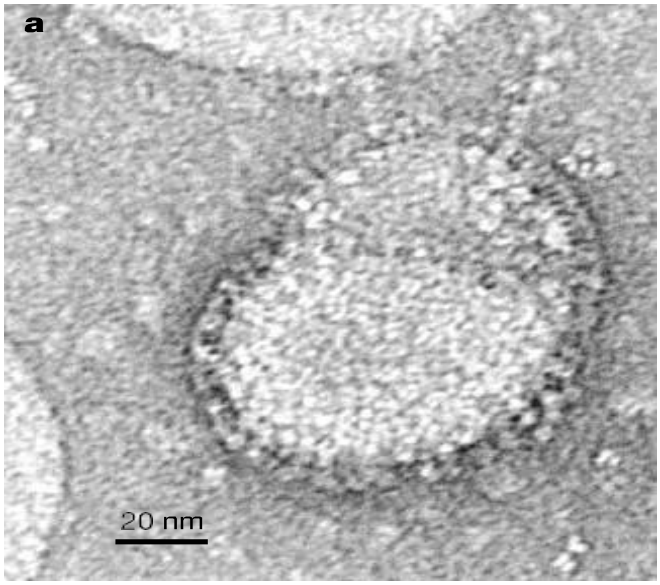
Dengue viruses, are categorized under the genus Flavivirus. These viruses contain single stranded RNA and are small in size (50 nm). Four dengue virus serotypes are designated as DENV-1, DENV-2, DENV-3 and DENV-4 .More than one serotype can be in circulation at a point of time.. Each offers cross protection only for a short period of time. Infection with any one serotype confers lifelong immunity to the virus serotype.

Fig. 2. Seasonal trends of Dengue/DHF 2003-07



Cocirculation of virus is possible because this doesn't offer protection in heteroantibodies. Individual variations occur in antibody responses to the dengue virus. Secondary infections are associated with elevated risks of severe disease outcomes. Primary and secondary infections are differentiated based on the antigenic responses. Superadded infection may cause worse infection , which is salient for dengue virus . All four serotypes are reported from India.

The dengue virus genome is composed of three structural protein genes encoding the nucleocapsid of core protein ©, a membrane associated protein (M), an envelope protein and seven non-structural (NS) proteins – NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5.. NS1 protein interacts with immune system and evokes T cell responses. NS1 protein is utilized as a diagnostic marker of the infection



Dengue viral infection is mostly asymptomatic. The exact causes of severity among some patients when there is interaction between agent and host are still not clearly understood. Infected people play a major role in introducing the dengue virus by their movement to newer areas.

Vector

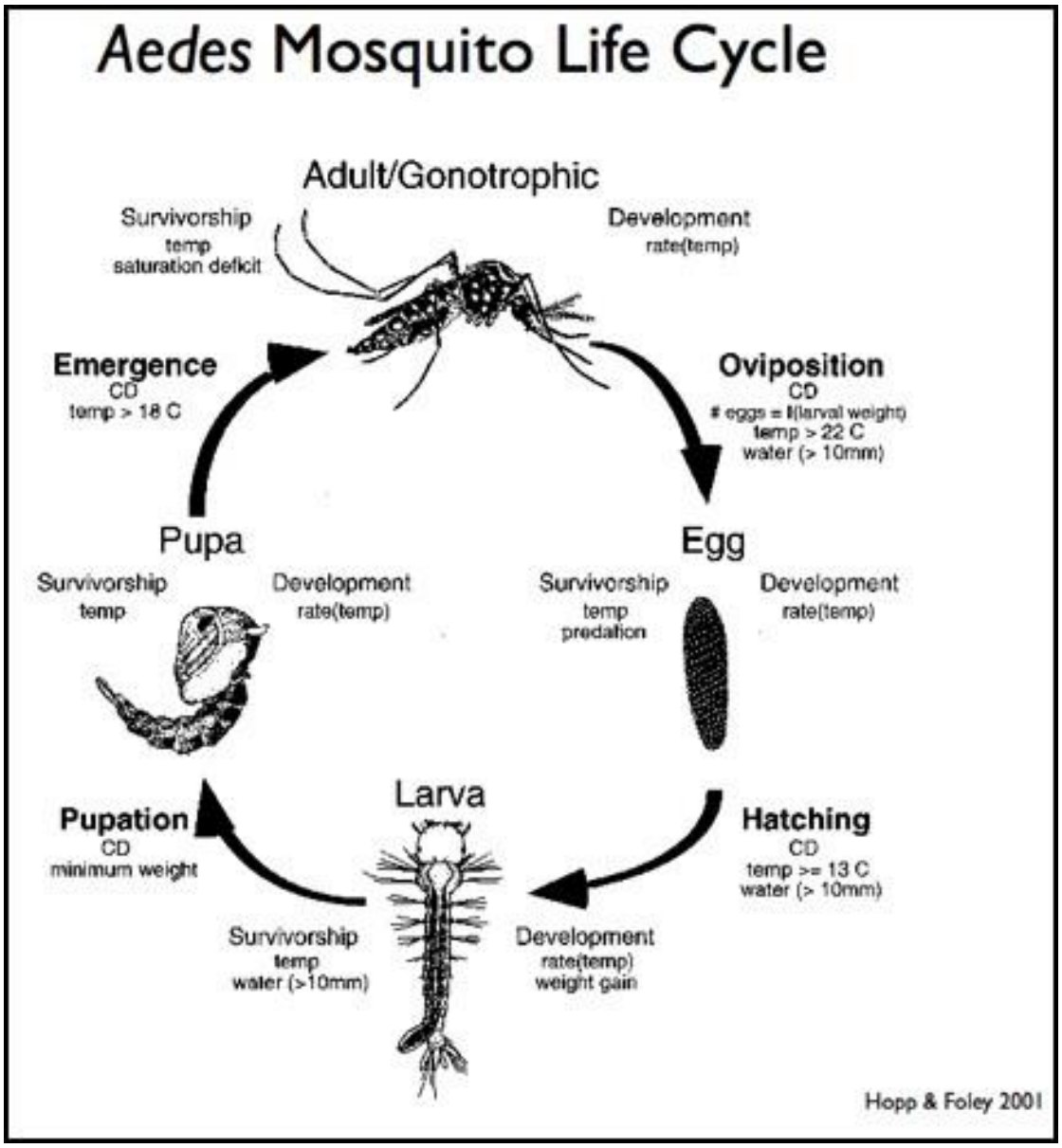
Aedes aegypti

Aedes aegypti habitats may be either terrestrial or aquatic which utilized tree holes and plant axils (leaf joints) found in forested areas as aquatic habitats historically. Adaptation of *Aedes aegypti* to urban domestic habitats have led to the exploitation of a range of artificial containers frequently associated with human habitation which include vases, water tanks and tyres.

The female *Aedes aegypti* feeds exclusively on humans and on a lower frequency of other hosts including bovine, swine, cat, rat, and chicken which represents <1% of bloodmeals

Aedes mates singly or in pairs , this plasticity allows for easy propagation of the mosquito “Aggregation pheromone” along with the olfactory stimulus and the wing beat rarte of the female aedes mosquito attracts the male mosquito and swarming occurs followed by mating .

Aedes aegypti is cited by the Global Invasive Species Database as the main vector in Yellow fever . cickungunya , west nile fever etc transmitted by *Aedes aegypti* together with the West Nile virus (European Centre for Disease Prevention and Control 2005-2015).



Control strategies are aimed at preventing mosquito bites, maintaining populations at “acceptable” densities, minimizing mosquito-host contact and reducing the longevity of female mosquitoes. It incorporates the concept of vector and disease control. Personal protection involves the use of domestic insecticides, repellents (natural or synthetic), insecticide treated materials and paints. Control also incorporates both biological and chemical methods (Fig. 5).

Transmission cycle

During a blood meal from an infected person Aedes gets affected and acquires the virus. Following an extrinsic incubation period of 8 to 10 days, the mosquito becomes infected .The mosquito transmits the virus via the saliva which it injects to prevent coagulation . The cycle of dengue continues by this process. Following an intrinsic incubation period of 4 to 7 days (range 3–14 days) Dengue begins abruptly. There is also evidence of vertical transmission of dengue virus from infected female mosquitoes to the next generation.

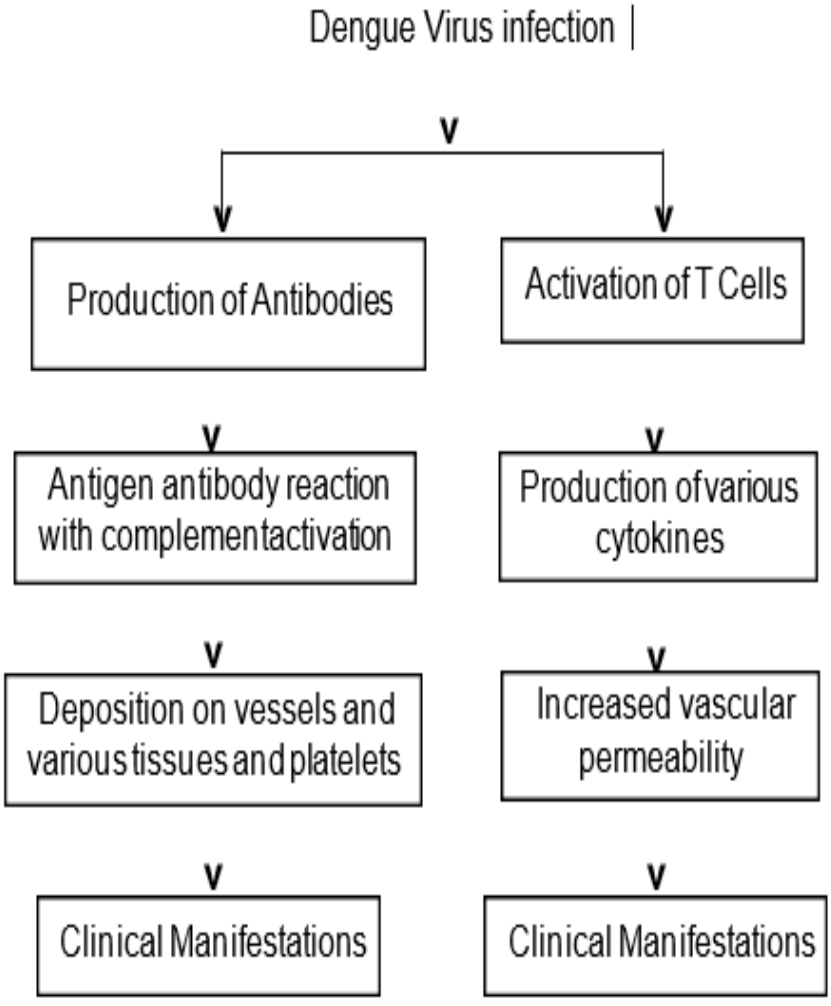
Vertical transmission and transmission by blood product transfusion have also been demonstrated.

Host factor

All ages and sexes are susceptible to infection . Fever within a week of return from a dengue endemic ares is highly suggestive of dengue . persistence of fever after 2 week of return from an endemic area is not suggestive of Dengue. The geographical spread of dengue has been reported to occur mainly by people travelling from endemic areas to non-endemic areas.

Genetic factors have been implicated in the occurrence of Dengue fever some are protective too. Certain HLA- class I and class II alleles, polymorphisms in the tumor necrosis factor alpha(TNF-α), Vitamin D receptor,[18] CTLA-4 and transforming growth factor ß (TGF-β)[19] have been shown to be associated with development of DHF/DSSSYMPTOMS:

Fig. 6. Patho-physiology of DHF



Host immune factors responsible for severe disease: cross reactive antibodies

Cross reactive T cells and antibodies are implicatedin the haphazard development of cytokine release and the exudation of fluid from th serosal surfaces , especially in secondary dengue syndrome .

Collectively, all this evidence suggests that the pathogenesis of DHF is far more complex than previously thought. Therefore, more detailed studies in patients with acute severe dengue infection and asymptomatic infection should be carried out in order to try and understand this complex immunopathogenesis.

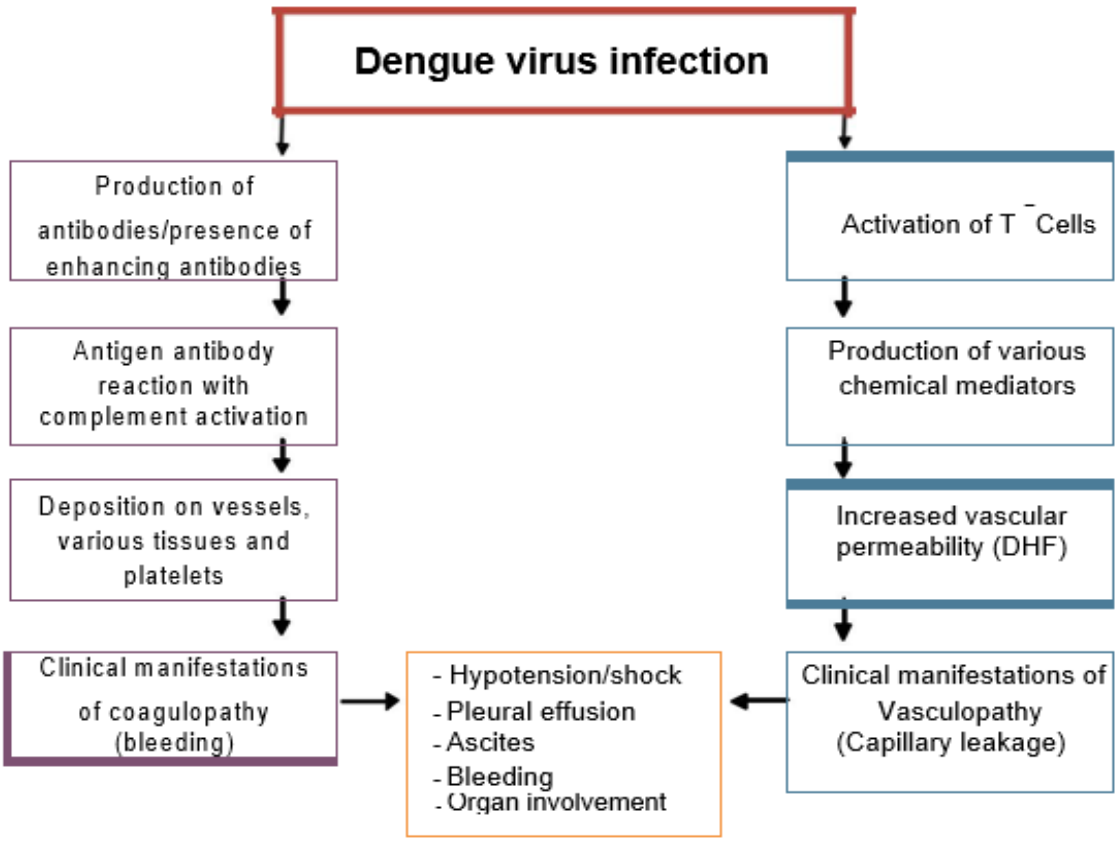
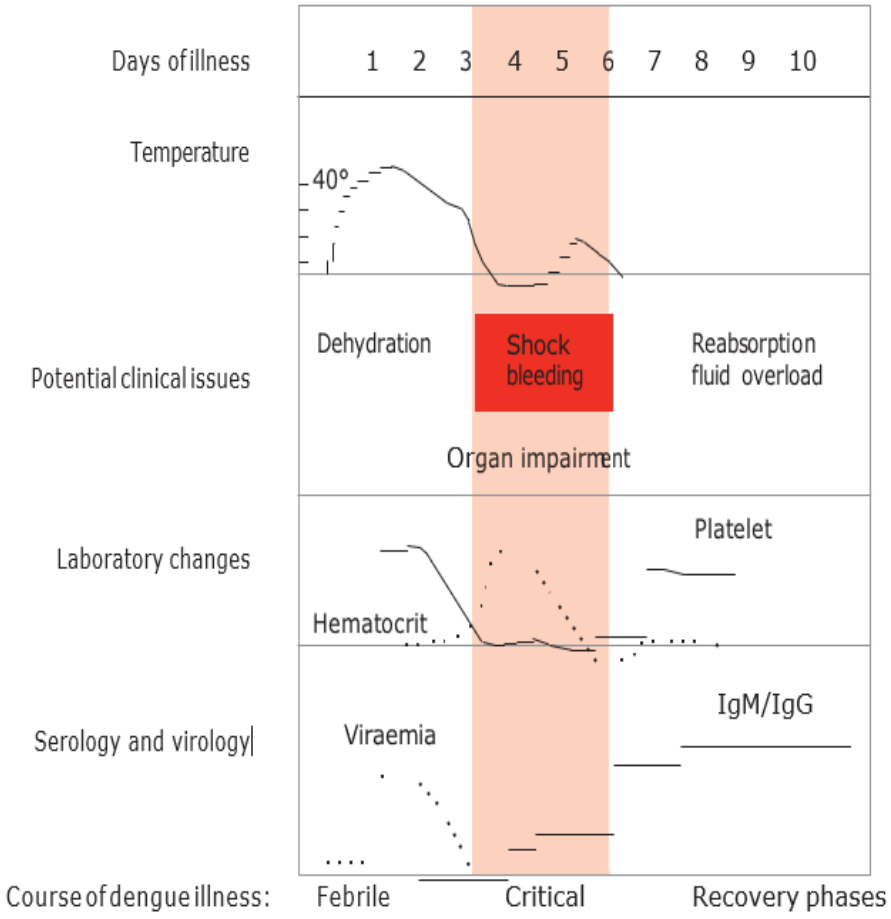


Fig. 4. Patho-physiology of DF/DHF

Dengue viral infected person may be asymptomatic or symptomatic and clinical manifestations vary from undifferentiated fever to florid haemorrhage and shock. The clinical presentations depend on various factors such as age, immune status of the host, the virus strain and primary or secondary infection. Infection with one dengue serotype gives lifelong immunity to that particular serotype.

Figure 2.1 The course of dengue illness*



* Source: adapted from Yip (2) by chapter authors.

Undifferentiated dengue Fever (UDF)

The fever of dengue is similar to any other virla illness and is highly difficult to differentiate from others . There may or may not be a rash during fever or defervescence . The symptoms of DF may not be very distinguished and signs of bleeding or capillary leakage may be absent.

Majority of the dengue virus infected persons are asymptomatic but symptomatic patients may present with undifferentiated fever, non-severe and severe manifestation. Some patients with dengue virus infection present with severe manifestations like shock, plasma leakage, bleeding and organ involvement. Based on thrombocyte count, haematocrit, evidence of capillary leakage, bleeding and hypotension. DHF has been divided into four grades. [15](Refer 3.8) Non severe cases may be DF and DHF grade I and II without significant bleeding. Severe dengue may be DHF III and IV with or without significant bleeding . DHF grade I and II may be severe when they present with significant bleeding or with metabolic and electrolyte abnormalities. Sometimes DF may present with life threatening significant bleeding without evidence of capillary leakage or haemoconcentration. Some dengue Fever patients may also present

with multiple organ involvement without bleeding and shock. In some patient there may be unusual atypical presentation also.

- Minor bleeding from different sites, scanty haemoptysis, haematemesis, haematuria, increase menstrual flow, gum bleeding, etc.
- Abdominal pain or discomfort
- Palpitation, breathlessness
- Hepatic dysfunction or hepatomegaly
- Decrease urinary output
- High HCT (>45%)
- Rapid fall in platelet count
- Cold clammy extremities
- Narrow pulse pressure
- Rapid pulse
- Hypotension

High Risk group

The following high risk groups may have severe manifestations or complications with DF/DHF, therefore this group of patients should be closely monitored for the development of severity:

- Pregnancy
- Infant
- Elderly
- Obesity
- Peptic ulcer diseases
- G6PD deficiency
- Thalassemia
- Coronary Artery Disease
- Chronic diseases: diabetes, COPD, bronchial asthma, hypertension
- Patients on steroid, antiplatelet, anticoagulant drugs
- HIV infected persons/ Immuno-compromised persons

Clinical Features of DF:

An acute febrile illness of 2-7 days duration with two or more of the following manifestations:

Headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestations.

System	Unusual or atypical manifestations
CNS involvement	Encephalopathy, encephalitis, febrile seizures, I/C bleed
G. I. involvement	Acute Hepatitis / fulminant hepatic failure, cholecystitis, cholangitis acute pancreatitis
Renal involvement	Acute renal failure, haemolytic uremic syndrome, acute tubular necrosis
Cardiac involvement	Cardiac arrhythmia, cardiomyopathy, myocarditis, pericardial effusion
Respiratory	Pulmonary oedema, ARDS, pulmonary haemorrhage. pleural effusion
Eye	Conjunctival bleed, macular haemorrhage, visual impairment, optic neuritis[16,17]

Dengue Haemorrhagic Fever (DHF):

a). A case with clinical criteria of dengue Fever plus

b). Haemorrhagic tendencies evidenced by one or more of the following

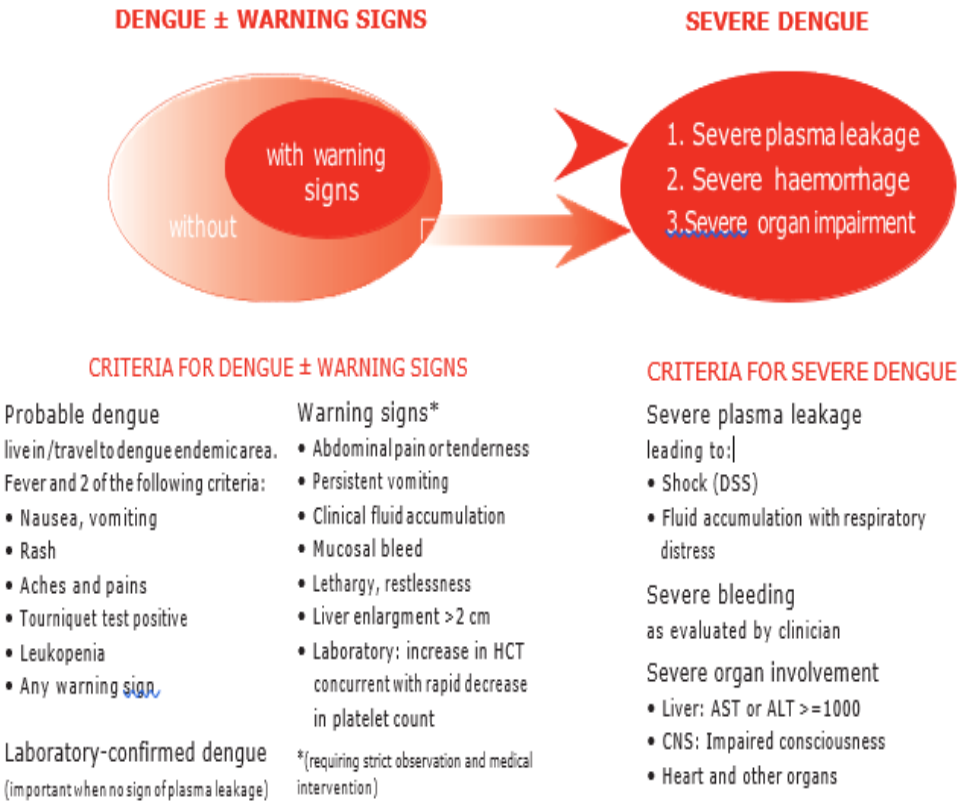
1. Positive tourniquet test
2. Petechiae, ecchymoses or purpura

3. Bleeding from mucosa, gastrointestinal tract, injection sites Plus

c). Thrombocytopenia (<100 000 cells per cumm) plus

d). Evidence of plasma leakage due to increased vascular permeability, manifested by one or more of the following:

1. A rise in average haematocrit for age and sex > 20%
2. A more than 20% drop in haematocrit following volume replacement treatment compared to baseline
3. Signs of plasma leakage (pleural effusion, ascites, hypoproteinemia)



Dengue Shock Syndrome (DSS):

All the above criteria for DHF with evidence of circulatory failure manifested by rapid and weak pulse and narrow pulse pressure (< 20% mm Hg) or hypotension for age, cold and clammy skin and restlessness.

Figure 5: Dengue patient with Maculopapular rash

Figure 6 : Impression mark on skin of a dengue patient

3.2.2 Severe dengue Fever

Probable DF/DHF:

A case compatible with clinical description (Clinical Criteria at 3.3) of dengue Fever during outbreak.:

OR

Non-ELISA based NS1 antigen/ IgM positive.

(A positive test by RDT will be considered as probable due to poor sensitivity and Specificity of currently available RDTs.)

Conifrmed dengue Fever:

A case compatible with the clinical description of dengue fever with at least one of the following

- Isolation of the dengue virus (Virus culture +VE) from serum, plasma, leucocytes.
- Demonstration of IgM antibody titre by ELISA positive in single serum
- Demonstration of dengue virus antigen in serum sample by NS1-ELISA.
- IgG seroconversion in paired sera after 2 weeks with Four fold increase of IgG titre.
- Detection of viral nucleic acid by polymerase chain reaction (PCR).

Natural course of dengue Infection

The clinical course of illness passes through the following three phases:

- Febrile phase
- Critical phase
- Convalescent phase

Febrile phase

Fever is biphasic with an appearance of a rash in the 2 to 7 th day associated with headache and nasal discharge . There may be pain in retro-orbital area, muscles, joint or bone. Fading rash is present during the 3rd to 5th day which is usually morbilliform and eruptive . Localized cluster of petechiae may appear over upper and lower limbs. Dengue Fever with unusual haemorrhagic manifestation may be seen rarely in case with co-morbid illness.

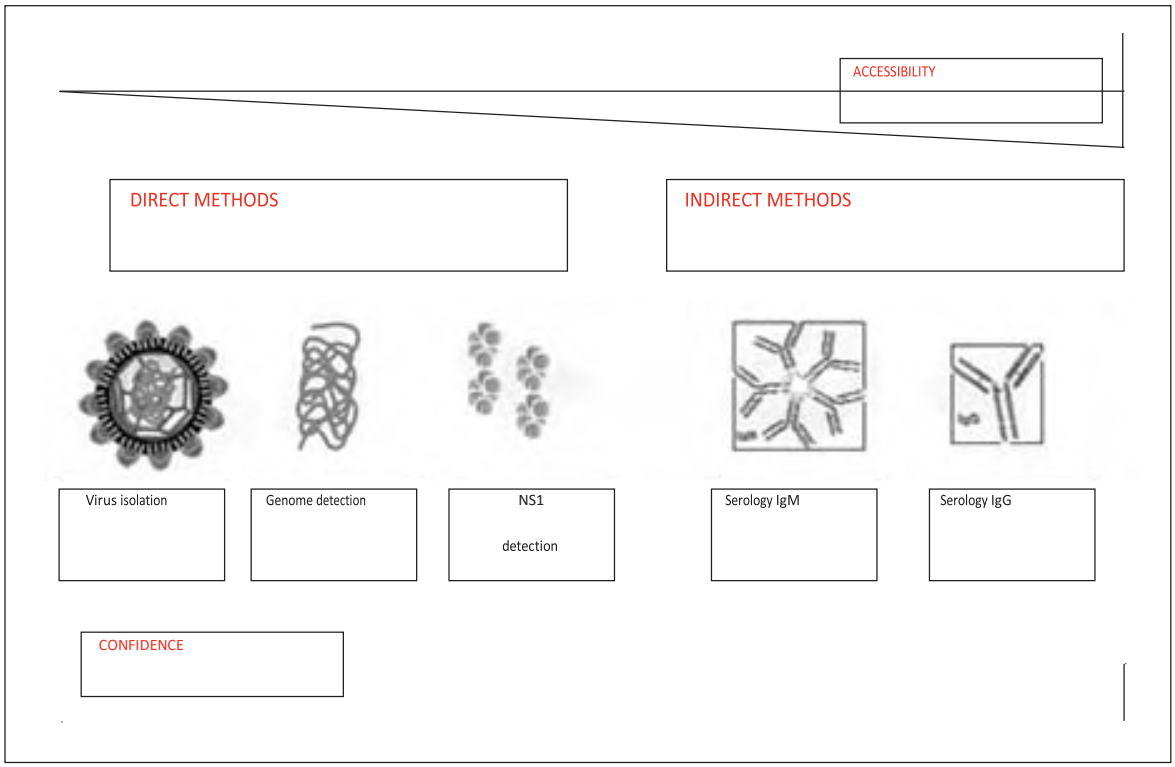
Critical phase (Leakage phase*)* 3 to 4 days after onset of fever DF/DHF patients usually go to critical phase. Plasma leakage and high haemoconcentration are documented during this critical phase and patients may develop hypotension. Shock, bleeding, accumulation of fluid in pleural and abdominal cavity are caused due to abnormal haemostasis and leakage of plasma. Various organ involvements and metabolic derangement causes High morbidity and mortality in DHF/DSS. This period of plasma leakage usually persists for 2 days .

Convalescent phase (recovery phase)

The extracellular fluid returns to the circulatory system during the recovery phase and signs and symptoms improve. After 6-7 days of fever one can expect this phase and it last for 2-3 days. Longer convalescence may be expected in some of the patients with severe shock, organ involvement and other complications which may require specific treatment. Pulmonary oedema may commence due to fluid overload if the fluid replacement is not optimized carefully.

Table 4.2
Methods for isolation of dengue virus

Method	Result confirming presence of dengue virus
Inoculation of mosquitos (adults or larvae)	Detection of antigen in head squash by serotype-specific immunofluorescence
Inoculation of various mammalian or insect cell cultures	Detection of antigen by antibody staining Cytopathic effect; identification of virus upon subpassage Plaque formation; identification of virus upon subpassage
Intracranial inoculation of suckling mice	Presence of antigen in brain detected by antibody staining Symptoms or signs indicating encephalitis Identification of virus upon subpassage



Laboratory diagnosis

Table 4.1 Summary of operating characteristics and comparative costs of dengue diagnostic n

Diagnostic methods	Diagnosis of acute infection	Time to results	Specimen	Time of collection after onset of symptoms	Facilities
Viral isolation and serotype identification	Confirmed	1–2 weeks	Whole blood, serum, tissues	1–5 days	Mosquito or cell culture facilities, BSL-2/BSL-3[a] laboratory, fluorescence microscope or molecular biology equipment
Nucleic acid detection	Confirmed	1 or 2 days	Tissues, whole blood, serum, plasma	1–5 days	BSL-2 laboratory, equipment for molecular biology
Antigen detection	Not yet determined Confirmed	1 day >1 day	Serum Tissue for immuno-chemistry	1–6 days NA	ELISA facilities Facilities for histology
IgM ELISA	Probable	1–2 days	Serum, plasma, whole blood	After 5 days	ELISA facilities
IgM rapid test		30 minutes			No additional supplies
IgG (paired	Confirmed	7 days	Serum, plasma,	Acute sera,	ELISA facilities

[a] Requirements may vary according to each country's national policies.

Kinetics of dengue virus replication and host response

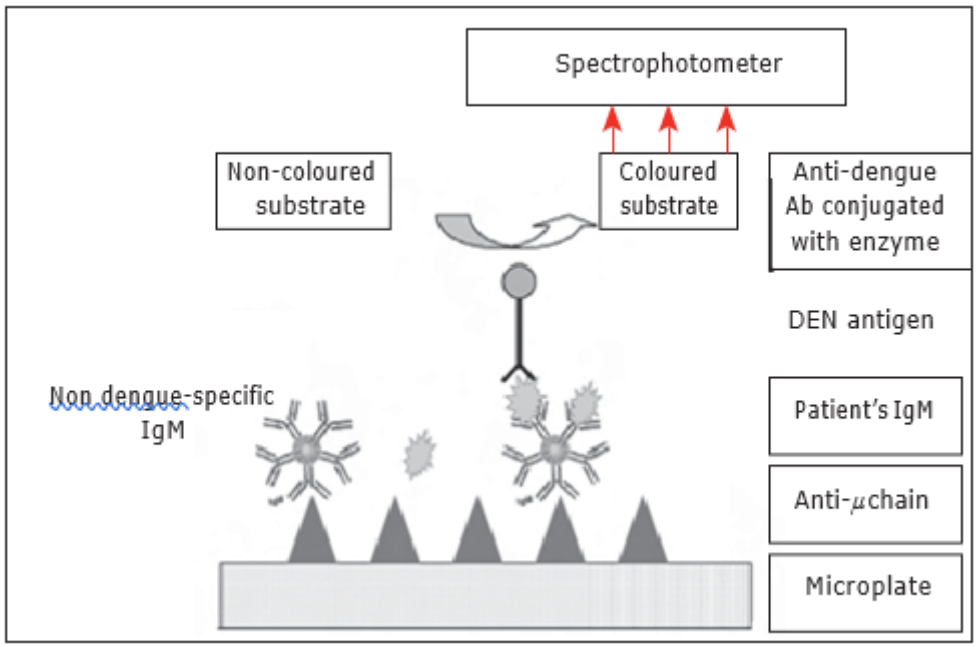
By the time a person infected with dengue virus develops fever, the infection is widely disseminated. The virus is found in serum or plasma, in circulating blood cells and in selected tissues, especially those of the immune system, for approximately 2–7 days, roughly corresponding to the period of fever.

In primary infection with dengue virus, serological tests may yield results that indicate a specific dengue serotype with specimens obtained early in the disease. In other cases, cross-reactive antibodies,

often apparent in the first 1– 2 months after infection, may confound determination of the serotype. In such cases, a monotypic antibody specific for the infecting serotype may be detected 3–6 months after infection. Therefore, specimens obtained during late conva- lescence from patients with a primary seroresponse pattern may be useful in determining the infecting dengue virus serotype.

Collection and handling of specimens

- Collect a specimen as soon as possible after the onset of illness, hospital admission or attendance at a clinic (this is called the acute serum, S1).
- Collect a specimen shortly before discharge from the hospital or, in the event of a fatality, at the time of death (convalescent serum, S2).
- Collect a third specimen, in the event hospital discharge occurs within 1–2 days of the subsidence of fever, 7–21 days after the acute serum was drawn (late convalescent serum, S3).



Specimen-collection procedures: tubes or vials

- Aseptically collect 2–5 ml or more of venous blood.
- Use adhesive tape marked with pencil or indelible ink or a typewritten or printed self-adhesive label to identify the container. At a minimum, the name of the patient, the identification number and the date of collection should be indicated.
- Use tubes or vials with screw-caps, if possible. Fix the cap with adhesive tape, wax or other sealing material to prevent leakage during transport.
- If a specimen cannot be analysed or shipped within 24 hours of being drawn, the serum should be separated from the cells and stored frozen.

- Ship specimens for culture or serology on wet ice to a laboratory as soon as possible.

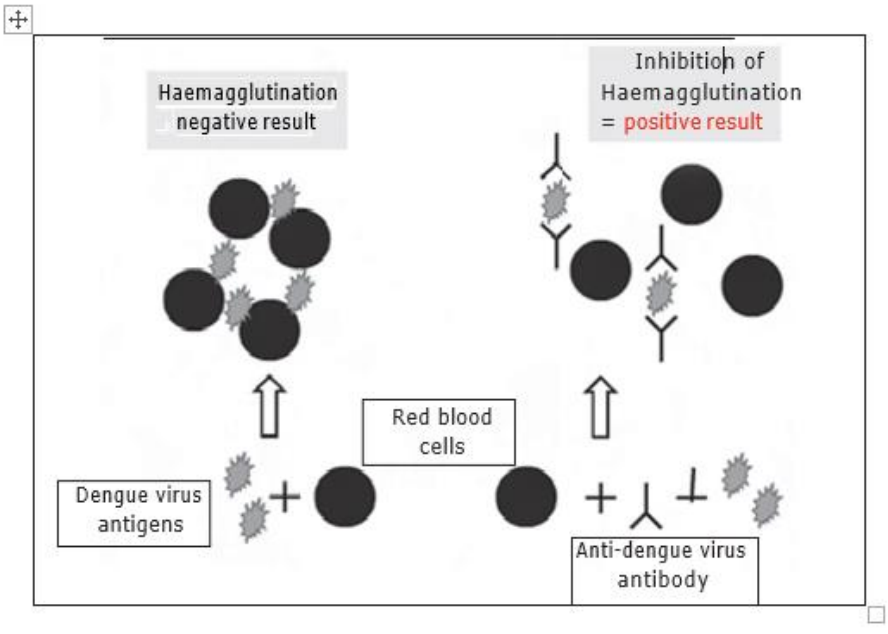
Serological tests

Interpretation of MAC-ELISA results[a]

IgM antibody response	S1–S2 interval[b]	IgM to IgG ratio	Interpretation
Increase in molar fraction	2–14 days	High Low	Acute flavivirus infection, primary Acute flavivirus infection, secondary
Elevated, no change or decrease in molar fraction	2–14 days	High Low	Recent flavivirus infection, primary Recent flavivirus infection, secondary
Elevated	(Single specimen)	High Low	Recent flavivirus infection, primary Recent flavivirus infection, probably secondary

Haemagglutination-inhibition test

Figure 4.4 Haemagglutination-inhibition assay



Dengue viruses agglutinate gander erythrocytes and those of certain other species as well as trypsinized type O human red blood cells. The HI test is based on the ability of dengue virus antibodies to inhibit this agglutination. The test is described in most virology manuals.

Interpretation of dengue haemagglutination-inhibition antibody response[a]

Antibody response	S1–S2 interval[b]	Convalescent titre[c]	Interpretation
≥4-fold rise	≤7 days	≥1:1280	Acute flavivirus infection, primary
≥4-fold rise	Any specimen	≥1:2560	Acute flavivirus infection, secondary
≥4-fold rise	<7 days	≥1:1280	Acute flavivirus infection, either primary or secondary
No change	Any specimen	>1:2560	Recent flavivirus infection, secondary
No change	≥7 days	≥1:1280	Not dengue
No change	<7 days	≥1:1280	Uninterpretable
Unknown	Single specimen	≥1:1280	Uninterpretable

Neutralization tests

Although several neutralization tests have been described for dengue virus, the most sensitive and specific method is the serum dilution, virus-constant, plaque-reduction test. Following primary dengue infection, relatively specific neutralizing antibodies are detected in early convalescence.

Dot-blot immunoassay

Dot-blot immunoassay technology is relatively new, and reagents and test procedures are evolving. At least one dot-blot immunoassay for dengue anti- bodies is available commercially. As greater interest develops among commer- cial manufacturers, additional dot-blot immunoassays are likely to enter the market.

Complement-fixation test

The complement-fixation test may also be used in serological diagnosis, al- though it is the least sensitive serological assay, and other assays have generally replaced this method. Complement-fixing antibody typically appears later than IgM or HI antibody and is usually more specific. Therefore, it can be useful in confirming dengue infection in patients with paired serum samples taken late in the infection. A fourfold rise in complement-fixing antibody, where the interval between the acute and convalescent serum is less than 2 weeks, signifies a secondary seroresponse pattern.

Isolation of dengue virus

Isolation of most strains of dengue virus from clinical specimens can be accomplished in the majority of cases, provided that the sample is taken

in the first five days of illness and processed without delay. Specimens that may be suitable for virus isolation include acute phase serum, plasma or washed buffy coat from the patient, autopsy tissues from fatal cases, especially liver, spleen, lymph nodes and thymus and mosquitoes collected in nature. Isolation of the virus takes 7–10 days, hence it may not be very useful for starting the management of patients with DF/DHF.

NVBDCP-recommended tests for laboratory diagnosis

–For confirmation of dengue infection, Government of India (GoI) recommends use of ELISA-based antigen detection test (NS1) for diagnosing the cases from the first day onwards and antibody detection test IgM capture ELISA (MAC-ELISA) for diagnosing the cases after the fifth day of onset of disease.[19]

–Directorate of National Vector Borne Disease Control Programme (NVBDCP), GoI has identified a network of laboratories (sentinel surveillance hospitals and apex referral laboratories) for surveillance of dengue fever cases across the country since 2007. These laboratories are also meant to augment the diagnostic facilities in all endemic areas. They are linked with Apex Referral Laboratories (ARLs) with advanced

diagnostic facilities for backup support and serotyping of dengue samples. For details, please refer to NVBDCP website www.nvbdcp.gov.in.

–These laboratories receive the samples, diagnose and send the report (line list) regularly to districts/municipal health authorities for implementation of preventive measures to interrupt the transmission.

–NS1 antigen tests – GoI introduced ELISA-based NS1 antigen test in 2010 in addition to MAC-ELISA tests which can detect the case during day 1 to day 5 of illness.

RECOMMENDATIONS FOR TREATMENT

Group A – patients who may be sent home (see the home care card for dengue in Textbox G)

These are patients who are able to tolerate adequate volumes of oral fluids and pass urine at least once every six hours, and do not have any of the warning signs, particularly when fever subsides.

Ambulatory patients should be reviewed daily for disease progression (decreasing white blood cell count, defervescence and warning signs) until they are out of the critical period. Those with stable haematocrit can be sent home after being advised to return

to the hospital immediately if they develop any of the warning signs and to adhere to the following action plan:

- Encourage oral intake of oral rehydration solution (ORS), fruit juice and other fluids containing electrolytes and sugar to replace losses from fever and vomiting. Adequate oral fluid intake may be able to reduce the number of hospitalizations (*13*). [Caution: fluids containing sugar/glucose may exacerbate hyperglycaemia of physiological stress from dengue and diabetes mellitus.]
- Give paracetamol for high fever if the patient is uncomfortable. The interval of paracetamol dosing should not be less than six hours. Tepid sponge if the patient still has high fever. Do not give acetylsalicylic acid (aspirin), ibuprofen or other non-steroidal anti-inflammatory agents (NSAIDs) as these drugs may aggravate gastritis or bleeding. Acetylsalicylic acid (aspirin) may be associated with Reye's Syndrome.

- Instruct the care-givers that the patient should be brought to hospital immediately if any of the following occur: no clinical improvement, deterioration around the time of defervescence, severe abdominal pain, persistent vomiting, cold and clammy extremities, lethargy or irritability/restlessness, bleeding (e.g. black stools or coffee-ground vomiting), not passing urine for more than 4–6 hours.

Patients who are sent home should be monitored daily by health care providers for temperature pattern, volume of fluid intake and losses, urine output (volume and frequency), warning signs, signs of plasma leakage and bleeding, haematocrit, and white blood cell and platelet counts (see group B).

Group B – patients who should be referred for in-hospital management

Patients may need to be admitted to a secondary health care centre for close observation, particularly as they approach the critical phase. These include patients with warning signs, those

with co-existing conditions that may make dengue or its management more complicated (such as pregnancy, infancy, old age, obesity, diabetes mellitus, renal failure, chronic haemolytic diseases), and those with certain social circumstances (such as living alone, or living far from a health facility without reliable means of transport).

- Obtain a reference haematocrit before fluid therapy. Give only isotonic solutions such as 0.9% saline, Ringer's lactate, or Hartmann's solution. Start with 5–7 ml/ kg/hour for 1–2 hours, then reduce to 3–5 ml/kg/hr for 2–4 hours, and then reduce to 2–3 ml/kg/hr or less according to the clinical response (Textboxes H, J and K).
- Reassess the clinical status and repeat the haematocrit. If the haematocrit remains the same or rises only minimally, continue with the same rate (2–3 ml/kg/hr) for another 2–4 hours. If the vital signs are worsening and haematocrit is rising rapidly, increase the rate to 5–10 ml/kg/hour for 1–2 hours. Reassess the clinical status, repeat the haematocrit and review fluid infusion rates

- 2) Give the minimum intravenous fluid volume required to maintain good perfusion and urine output of about 0.5 ml/kg/hr. Intravenous fluids are usually needed for only 24–48 hours. Reduce intravenous fluids gradually when the rate of plasma leakage decreases towards the end of the critical phase. This is indicated by urine output and/or oral fluid intake that is/are adequate, or haematocrit decreasing below the baseline value in a stable patient.
- Patients with warning signs should be monitored by health care providers until the period of risk is over. A detailed fluid balance should be maintained. Parameters that should be monitored include vital signs and peripheral perfusion (1–4 hourly until the patient is out of the critical phase), urine output (4–6 hourly), haematocrit (before and after fluid replacement, then 6–12 hourly), blood glucose, and other organ functions (such as renal profile, liver profile, coagulation profile, as indicated).

Group C – patients who require emergency treatment and urgent referral when they have severe dengue

Patients require emergency treatment and urgent referral when they are in the critical phase of disease, i.e. when they have:

– severe plasma leakage leading to dengue shock and/or fluid accumulation with respiratory distress;

– severe haemorrhages;

– severe organ impairment (hepatic damage, renal impairment, cardiomyopathy, encephalopathy or encephalitis).causes such as essential thrombocytosis, polycythemia vera, protein C and S deficiency.

- Start intravenous fluid resuscitation with isotonic crystalloid solutions at 5–10 ml/kg/hour over one hour. Then reassess the patient's condition (vital signs, capillary refill time, haematocrit, urine output). The next steps depend on the situation.

- If the patient's condition improves, intravenous fluids should be gradually reduced to 5–7 ml/kg/hr for 1–2 hours, then to 3–5 ml/kg/hr for 2–4 hours, then to 2–3 ml/kg/hr, and then further depending on haemodynamic status, which can be maintained for up to 24–48 hours. (See textboxes H and J for

a more appropriate estimate of the normal maintenance requirement based on ideal body weight).

- If vital signs are still unstable (i.e. shock persists), check the haematocrit after the first bolus. If the haematocrit increases or is still high (>50%), repeat a second bolus of crystalloid solution at 10–20 ml/kg/hr for one hour. After this second bolus, if there is improvement, reduce the rate to 7–10 ml/ kg/hr for 1–2 hours, and then continue to reduce as above. If haematocrit decreases compared to the initial reference haematocrit (<40% in children and adult females, <45% in adult males), this indicates bleeding and the need to cross-match and transfuse blood as soon as possible (see treatment for haemorrhagic complications).
- Further boluses of crystalloid or colloidal solutions may need to be give

Treatment of complications and other areas of treatment

Fluid overload with large pleural effusions and ascites is a common cause of acute respiratory distress and failure in severe dengue. Other causes of respiratory distress include acute pulmonary

oedema, severe metabolic acidosis from severe shock, and Acute Respiratory Distress Syndrome (ARDS) (refer to standard textbook of clinical care for further guidance on management).

Causes of fluid overload are:

– excessive and/or too rapid intravenous fluids;

– incorrect use of hypotonic rather than isotonic crystalloid solutions;

– inappropriate use of large volumes of intravenous fluids in patients with unrecognized severe bleeding;

– inappropriate transfusion of fresh-frozen plasma, platelet concentrates and cryoprecipitates;

– continuation of intravenous fluids after plasma leakage has resolved (24–48 hours from defervescence);

– co-morbid conditions such as congenital or ischaemic heart disease, chronic lung and renal diseases.

Chart 1. Volume replacement algorithm for patients with DHF grades I & II

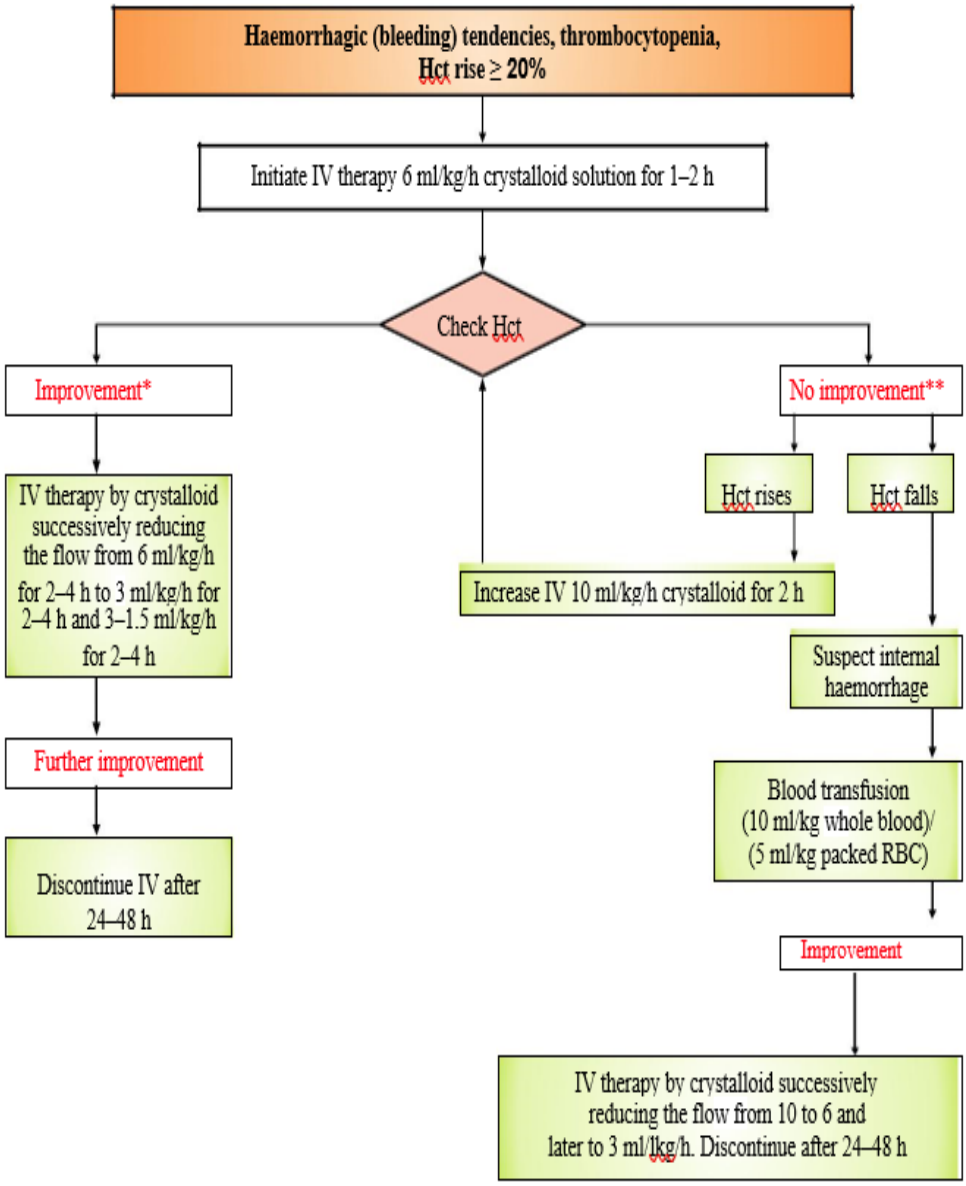
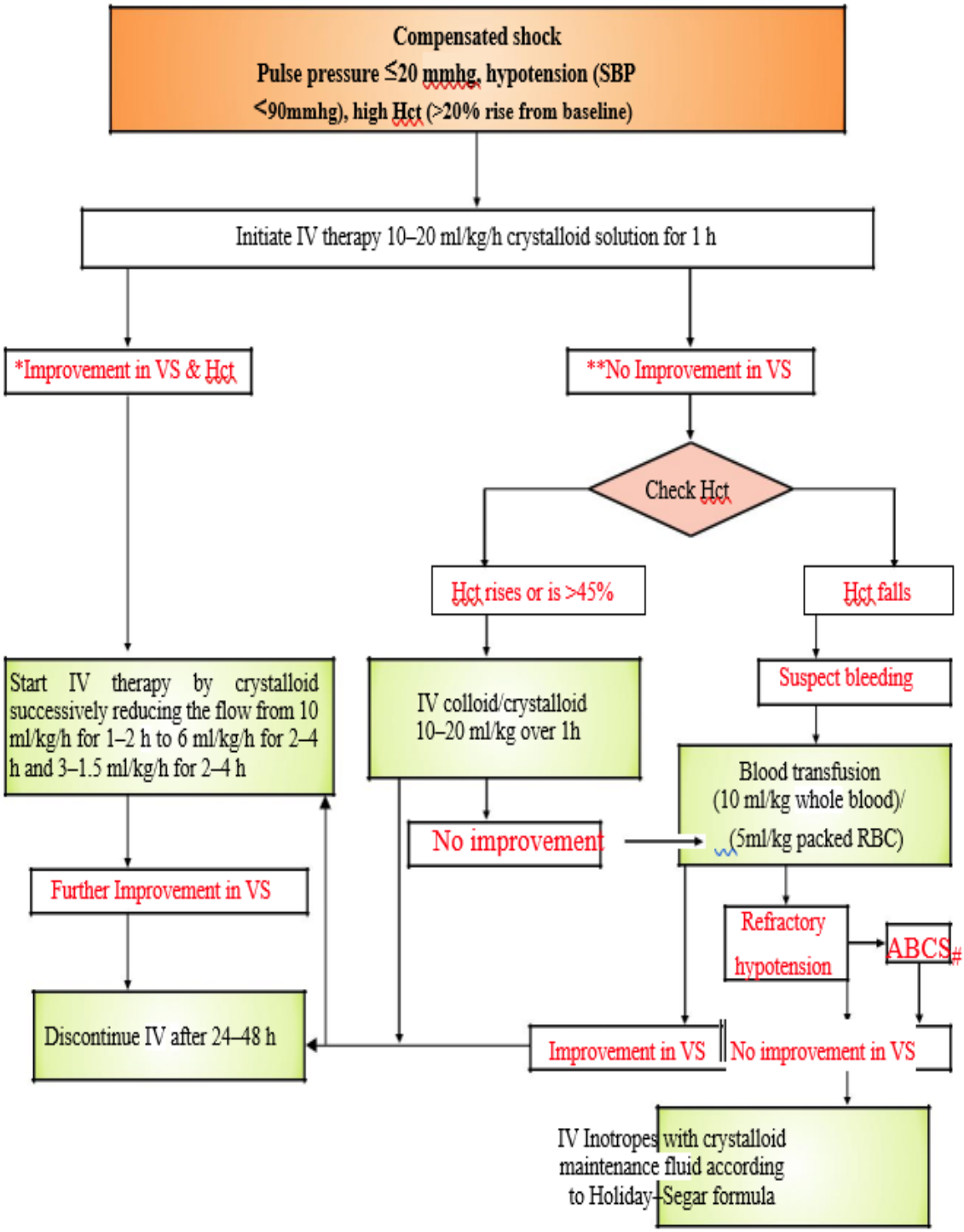


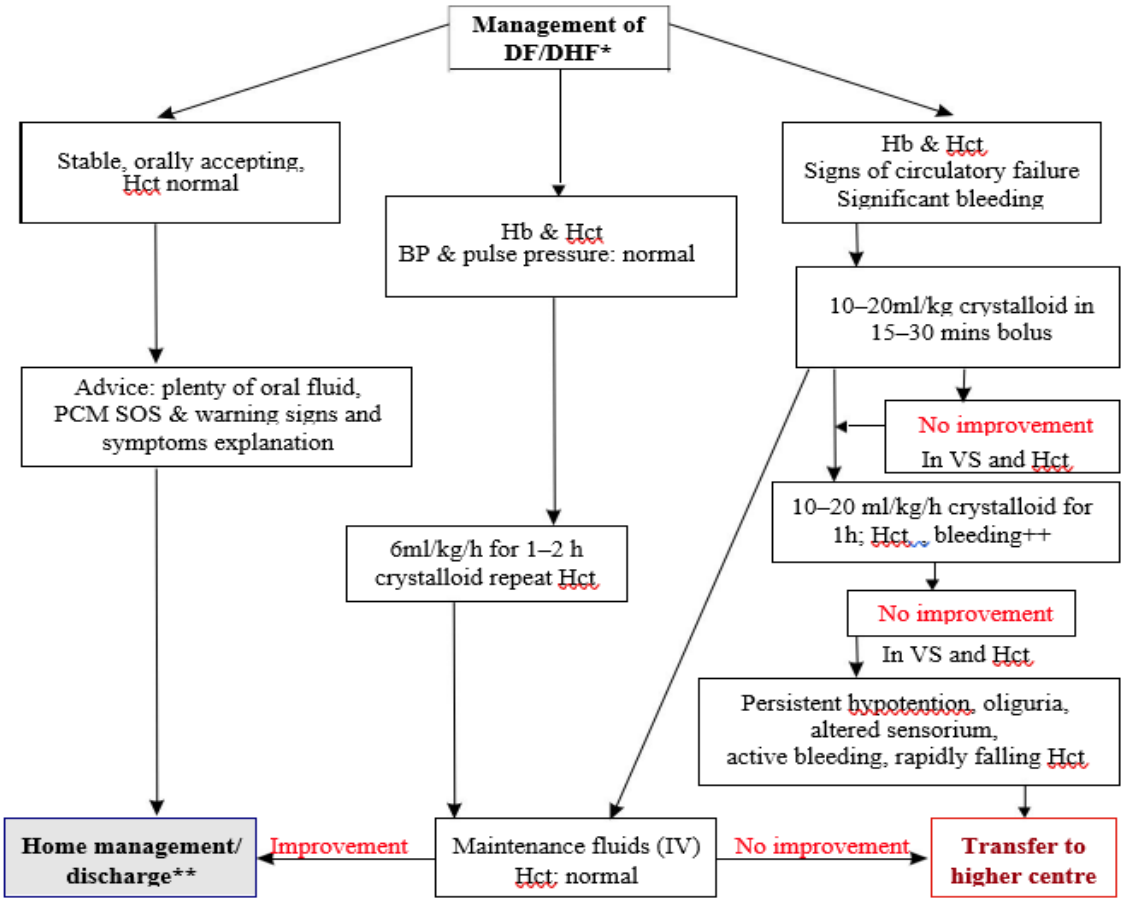
Chart 2. Volume replacement algorithm for patients with DHF grade III



.Early clinical features of fluid overload are:

- respiratory distress, difficulty in breathing;
- rapid breathing;
- chest wall in-drawing;
- wheezing (rather than crepitations);
- large pleural effusions;
- tense ascites;
- increased jugular venous pressure (JVP).

Fig. 8. Guidelines to be followed in the PHC for management of Dengue cases



*Look for co-morbid illnesses and coinfections – refer Sections 5.3 and 5.4 for details

Late clinical features are:

– pulmonary oedema (cough with pink or frothy sputum ± crepitations, cyanosis);

– irreversible shock (heart failure, often in combination with ongoing hypovolaemia).

Additional investigations are:

– the chest x-ray which shows cardiomegaly, pleural effusion, upward displacement of the diaphragm by the ascites and varying degrees of “bat’s wings” appearance ± Kerley B lines suggestive of fluid overload and pulmonary oedema;

– ECG to exclude ischaemic changes and arrhythmia;

– arterial blood gases;

– echocardiogram for assessment of left ventricular function, dimensions and regional wall dyskinesia that may suggest underlying ischaemic heart disease;

– cardiac enzymes.even before ascites may develop.

The action plan for the treatment of fluid overload is as follows:

- Oxygen therapy should be given immediately.
- Stopping intravenous fluid therapy during the recovery phase will allow fluid in the pleural and peritoneal cavities

to return to the intravascular compartment. This results in diuresis and resolution of pleural effusion and ascites. Recognizing when to decrease or stop intravenous fluids is key to preventing fluid overload. When the following signs are present, intravenous fluids should be discontinued or reduced to the minimum rate necessary to maintain euglycaemia:

- signs of cessation of plasma leakage;
- stable blood pressure, pulse and peripheral perfusion;
- haematocrit decreases in the presence of a good pulse volume;
- afebrile for more than 24–48 days (without the use of antipyretics);
- resolving bowel/abdominal symptoms;
- improving urine output.

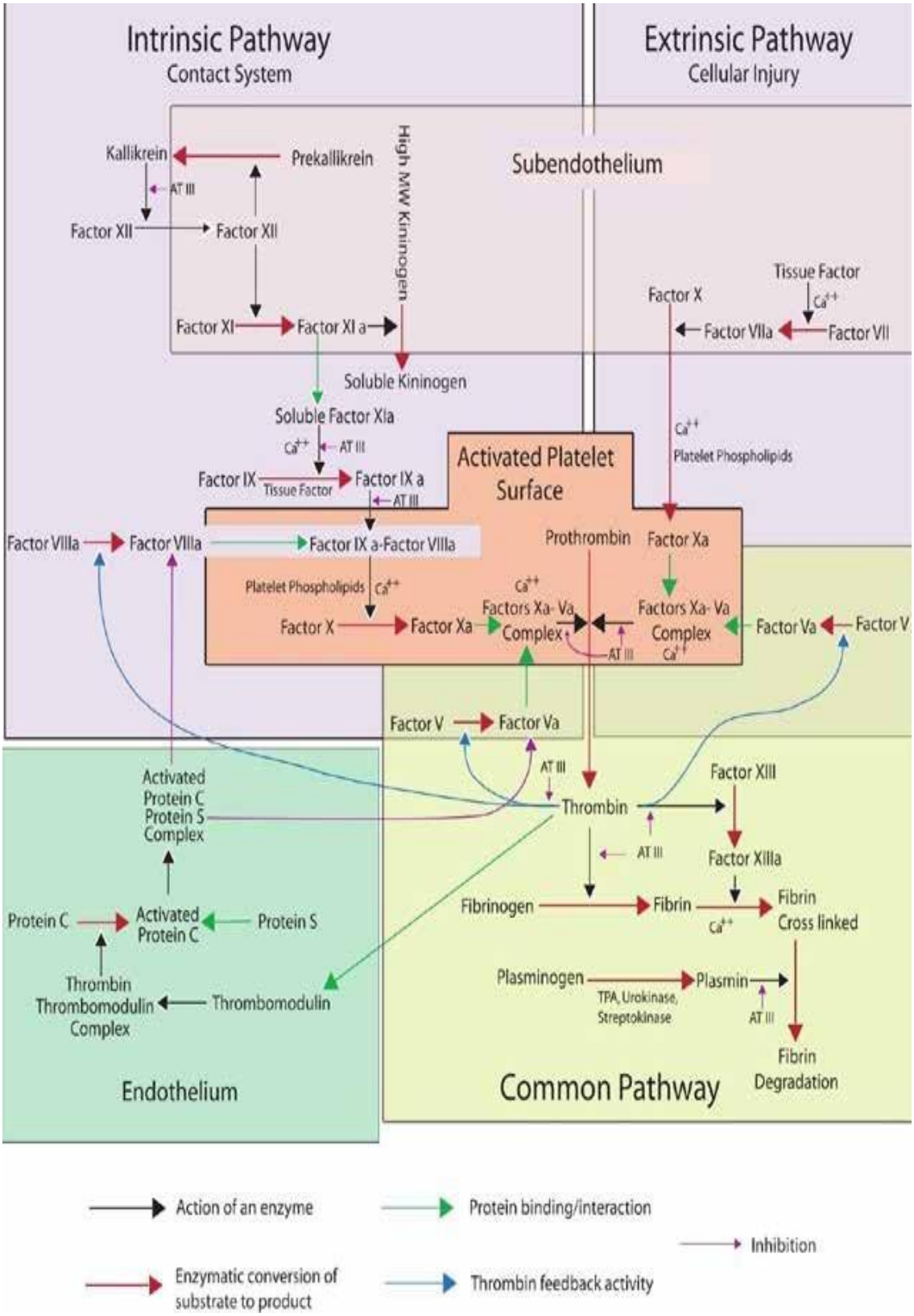
- The management of fluid overload varies according to the phase of the disease and the patient's haemodynamic status. If the patient has stable haemodynamic status and is out of the critical phase (more than 24–48 hours of defervescence), stop intravenous fluids but continue close monitoring. If

necessary, give oral or intravenous furosemide 0.1–0.5 mg/kg/dose once or twice daily, or a continuous infusion of furosemide 0.1 mg/kg/hour. Monitor serum potassium and correct the ensuing hypokalaemia.

- If the patient has stable haemodynamic status but is still within the critical phase, reduce the intravenous fluid accordingly. Avoid diuretics during the plasma leakage phase because they may lead to intravascular volume depletion.
- Patients who remain in shock with low or normal haematocrit levels but show signs of fluid overload may have occult haemorrhage. Further infusion of large volumes of intravenous fluids will lead only to a poor outcome. Careful fresh whole blood transfusion should be initiated as soon as possible. If the patient remains in shock and the haematocrit is elevated, repeated small boluses of a colloid solution may help.

Coagulation Cascade

1. “Primary haemostasis(vasoconstriction and platelet plug formation)
2. Secondary haemostasis (activation of coagulation factors and generation of thrombin)
3. Fibrin clot formation and stabilization
4. Inhibition of coagulation (inhibition of thrombin generation and fibrin clot breakdown)


Intrinsic Pathway
Contact System
Extrinsic Pathway
Cellular Injury
Subendothelium
Kallikrein
Prekallikrein
AT III
Factor XII
Factor XII
High MW Kininogen
Factor XI
Factor XI a
Soluble Kininogen
Soluble Factor XIa
Ca++
AT III
Factor IX
Tissue Factor
Factor IX a
AT III
Factor X
Tissue Factor
Ca++
Factor VIIa
Factor VII
Ca++
Platelet Phospholipids
Activated Platelet Surface
Factor VIIIa
Factor VIIIa
Factor IX a-Factor VIIIa
Prothrombin
Factor Xa
Platelet Phospholipids
Ca++
Ca++
Factor X
Factor Xa
Factors Xa- Va Complex
Factors Xa- Va Complex
Factor Va
Factor V
AT III
Ca++
Factor V
Factor Va
AT III
Factor XIII
Activated Protein C Protein S Complex
Thrombin
AT III
Factor XIIIa
Protein C
Activated Protein C
Protein S
Fibrinogen
Fibrin
Ca++
Fibrin Cross linked
Thrombin Thrombomodulin Complex
Thrombomodulin
Plasminogen
Plasmin
TPA, Urokinase, Streptokinase
AT III
Fibrin Degradation
Endothelium
Common Pathway
Action of an enzyme
Protein binding/interaction
Inhibition
Enzymatic conversion of substrate to product
Thrombin feedback activity

The prothrombin time (PT) measures the integrity of extrinsic and common pathways of coagulation (factors VII, X and V; prothrombin and fibrinogen). The activated partial thromboplastin time (aPTT) measures the integrity of the intrinsic and common pathways of coagulation (high molecular weight kininogen; prekallikrein; factors XII, XI, IX, VIII, X and V; prothrombin and fibrinogen). The sensitivity of the PT and aPTT in detecting coagulation factors deficiencies may vary with the reagent used to perform these tests, and each laboratory must determine its own reference standards. The thrombin time (TT) is a screen for quantitative deficiencies and qualitative defects of plasma fibrinogen.

Thromboelastography (TEG) assesses the viscoelastic properties of blood samples under low shear conditions. It measures the clot's physical property by using a stationary cylindrical cup that hold the blood sample and oscillates through an angle of 4o45 with each rotation cycle lasting 10 sec. Besides standard TEG, Platelet Mapping which is an extension of TEG technology, in addition to providing information on clot formation and lyses of whole blood sample, it quantifies the contribution of fibrin, adenosine diphosphate (ADP) receptor and thromboxane A2 (Tx A2) receptor in clot

Prothrombin Time (PT)

Principle: The prothrombin time test belongs to a group of blood tests that assess the clotting ability of blood.4, 5 The test is also known as the pro time or PT test. The blood is collected in a tube that contains sodium citrate to prevent the clotting process from starting before the test. The blood cells are separated from the liquid part of blood (plasma). The PT test is performed by adding the patient's plasma to a protein in the blood (thromboplastin) that converts prothrombin to thrombin. The mixture is then kept in a warm water bath at 37°C for one to two minutes. Calcium chloride is added to the mixture in order to counteract the Sodium citrate and allow clotting to proceed. The test is timed from the addition of the calcium chloride until the plasma clots. This time is called as the prothrombin time.

Normal value : The normal prothrombin time is 11-15 seconds. A prothrombin time within this range indicates that the patient has normal amounts of clotting factors VII and X. A prolonged PT time is considered abnormal.

Abnormal value: The prothrombin time will be prolonged if the concentration of any of the tested factors is 10% or more below normal plasma values. A prolonged prothrombin time indicates a deficiency in any of factors VII, X, V, prothrombin, or fibrinogen. It may mean that the

patient has a vitamin K deficiency, a liver disease, or disseminated intravascular coagulation (DIC). The prothrombin time of patients receiving warfarin therapy will also be prolonged-usually in the range of one and one half to two times the normal PT time. PT time that exceeds approximately two and a half times the control value (usually 30 seconds or longer) is ground for concern, as abnormal bleeding may occur.

Limitations: Spurious results may occur if the blood to anticoagulant ratio is not 9:1. The PT clotting times may be prolonged by substances including corticosteroids, EDTA, oral contraceptives, asparaginase, clofibrate, erythromycin, ethanol, Tetracycline and anticoagulants such as heparin and coumarin. The PT may be shortened by substances including antihistamines, butabarbital

Activated Partial Thromboplastin Time (APTT)

Principle : APTT is the time required for plasma to be clotted when maximal surface contact activation and optimal phospholipid and calcium concentration are provided.

Results are expressed as time seconds or as ratio.

Normal range: Every laboratory should determine its own normal range. In our laboratory with commercial reagents it is 29-35 seconds.

Interpretation : APTT is sensitive to the deficiencies or abnormalities of both intrinsic and common coagulation factors *i.e.* Factors I, II, V, X, VIII,

IX, XI, XII, Fletcher factor and Fitzgerald factor. The test is more sensitive to an abnormality occurring in the early stage of coagulation mechanism *i.e.* factors leading upto the generation of Factor Xa and less sensitive to later stage *i.e.* Factor II fibrinogen. A deficiency levels < 20-50% of Factor XII, XI, IX, XIII, X or V would give prolonged APTT. Where as a deficiency upto 10% of Prothrombin and 0.5-1g / litre of fibrinogen or less would give an abnormal APTT.

APTT is also prolonged when there is an inhibitor present in patient's plasma. Therefore whenever a prolonged APTT is detected, screening test for inhibitor must be performed. It is also an important test for the control of heparin therapy. Therapeutic range-60-100 seconds.

APTT Mixing Study with normal serum / adsorbed plasma

Principle: Plasma samples found to have a prolonged APTT are further investigated to define the abnormality by performing mixing or correction tests. Correction of the abnormality by the additive indicates that the reagent must contain the substance deficient in the test sample. An abnormal APTT is repeated on 50:50 mixtures of a known congenitally deficient plasma and the test plasma, or on 50:50 mixtures of aged / adsorbed plasma and test plasma until correction is obtained and the missing factor identified.

MILD, MODERATE AND SEVERE ELEVATIONS OF AMINOTRANSFERASES

1. **Severe (> 20 times, 1000 U/L) :** The AST and ALT levels are increased to some extent in almost all liver diseases. The highest elevations occur in severe viral hepatitis, drug or toxin induced hepatic necrosis and circulatory shock. Although enzyme levels may reflect the extent of hepatocellular necrosis they do not correlate with eventual outcome. In fact declining AST and ALT may indicate either recovery of poor prognosis in fulminant hepatic failure.4, 5
2. **Moderate (3-20 times):** The AST and ALT are moderately elevated in acute hepatitis, neonatal hepatitis, chronic hepatitis, autoimmune hepatitis, drug induced hepatitis, alcoholic hepatitis and acute biliary tract obstructions. The ALT is usually more frequently increased as compared to AST except in chronic liver disease. In uncomplicated acute viral hepatitis, the very high initial levels approach normal levels within 5 weeks of onset of illness and normal levels are obtained in 8 weeks in 75% of cases.

For reasons, which are not, understood AST levels appear disproportionately low in patients with Wilson disease.4,5

3. **Mild (1-3 times) :** These elevations are usually seen in sepsis induced neonatal hepatitis, extrahepatic biliary atresia (EHBA),

fatty liver, cirrhosis, non alcoholic steato hepatitis(NASH), drug toxicity, myositis, duchenne muscular dystrophy and even after vigorous exercise.1,4

One third to one half of healthy individuals with an isolated elevation of ALT on repeated testing have been found to be normal.13

AST: ALT ratio

The ratio of AST to ALT is of use in Wilson disease, CLD and alcoholic liver disease and a ratio of more than 2 is usually observed. The lack of ALT rise is probably due to pyridoxine deficiency. In NASH the ratio is less than one in the absence of fibrosis on liver biopsy.4

In viral hepatitis the ratio is usually less than one. The ratio invariably rises to more than one as cirrhosis develops possibly because of reduced plasma clearance of AST secondary to impaired function of sinusoidal cells.14

ALT exceeds AST in toxic hepatitis, viral hepatitis, chronic active hepatitis and cholestatic hepatitis 5

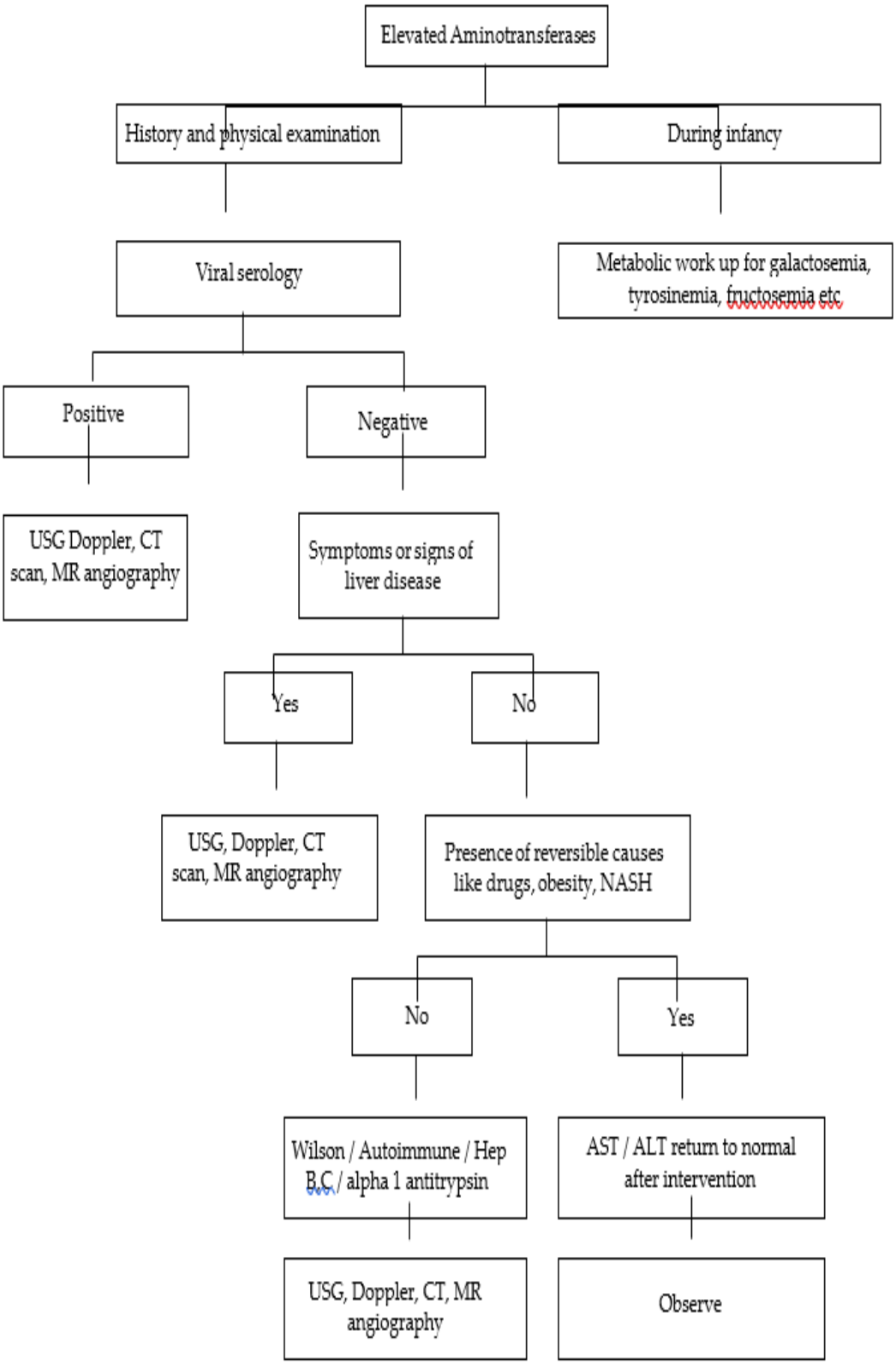


Fig. 2. Algorithm to Approach Mild but Sustained Rise of Aminotransferases

Biochemical Patterns of Liver Involvement in Dengue Virus Infections

Multiple investigators have documented elevated serum liver enzymes with dengue infections, particularly the transaminases Aspartate Transaminase (AST) and Alanine Transaminase (ALT). Kuo et al. published a series of 270 dengue patients and found elevated ALT and AST levels in 82% and 93%, respectively [9]. Most AST and ALT elevations were mild to moderate but elevations greater than ten times the upper limit of normal were seen in 11.1% and 7.4%, respectively. In a study of 169 serologically confirmed dengue cases in Rio de Janeiro, Brazil, 65.1% (110/169) had abnormal aminotransferases [17].

During acute infections, it is often not possible to do a liver biopsy due to thrombocytopenia, coagulation disturbances and/or the presence of ascites. Therefore, most of the published histological data on dengue-associated liver dysfunction comes from autopsy specimens. As a result, these data are skewed towards the more severe end of the disease spectrum. Histologic patterns of liver disease in milder cases are not clear. The available histologic data describe hepatocellular necrosis, apoptosis-induced Councilman Bodies, steatosis, and inflammatory cell infiltrates. Hepatocellular necrosis seen in dengue affects the midzonal and centrilobular areas of the hepatic acinus, which are the area's most susceptible to anoxia. Fatal Dengue Hemorrhagic Fever (DHF) autopsy

findings are characterized by the presence of variable degrees of hepatocyte necrosis, primarily midzonal. Data on the ability of elevated aminotransferase levels to predict dengue infection severity are conflicting. In a study of 690 dengue patients from Singapore, AST or ALT levels did not discriminate between DF and DHF or between non-severe and severe dengue [18]. However, median AST and ALT levels were significantly higher with increasing dengue severity by both 1997 and 2009 WHO criteria. Prakash et al. published a series of 699 dengue patients from Karachi, Pakistan [26]. In this study, the overall mortality was 33% in the mild to moderate hepatitis group and 67% in the severe hepatitis group ($p<0.002$). Severe hepatitis, defined as an ALT level greater than 300 IU/L, was associated with prolonged length of hospital stay, mortality, bleeding, and renal failure.

Aptt prolongation in dengue

Recently it has shown that non-structural protein 1 (NS1) of dengue virus can bind both to thrombin and pro thrombin. Binding to thrombin will not make any changes whereas pro thrombin activation is inhibited. This can explain changes in aPTT occur early before antibodies are formed.[3,4] NS1 also contribute plasma leakage by producing endothelial changes independent of immunological mechanism.[5,6] During acute phase of dengue lot of inflammatory mediators and cytokines are released. Complements like C3a C5a IL6, IL10, TNF α interferon ∞and histamine

leading to plasma leakage, thrombocytopenia, and decrease in fibrinogen coagulation factors. In addition, the inflammation hepatocytes leading to increase in AST, ALT. Damage to liver cells further decreases the coagulation factor synthesis this can alter the PT APTT, systems. Various studies have shown the participation of DV in the down regulation of the thrombomodulin-thrombin-protein C complex formation at the endothelial surface, with a reduction in activated protein C (APC). APC is the most important vasoprotective protein because it down regulates thrombin generation (by the inactivation of procoagulant factors Va and VIIIa) and has anti-inflammatory, ant apoptotic, and barrier protection properties. These biological functions of APC are associated with the endothelial protein C receptor (EPCR) and protease-activated receptor 1 (PAR-1) signalling pathways, which link the coagulation-inflammation responses. Alterations in the antithrombotic and cyto protective protein C pathways during DV infection of human endothelial vascular cells have been observed, which may explain the vasculopathy observed during DHF

MATERIALS AND METHODS

STUDY POPULATION:

The study will be conducted on 100 dengue patients admitted to GRH , Madurai during the study period of 12 months

INCLUSION CRITERIA:

- Patients presenting with fever from a dengue endemic
- H/o headache , joint pain , nause , vomiting
- lab confirmed dengue
- positive tourniquet test

EXCLUSION CRITERIA:

- Patients with bleeding diathesis .
- Patients on Anticoagulant therapy .
- Alcoholics .
- CLD patients

DATA COLLECTION:

A previously designed proforma was used to collect the demographic and clinical details of the patients. All the patients underwent detailed clinical evaluation, appropriate investigations,

STUDY PROTOCOL:

DESIGN OF STUDY:

- **Prospective study**

PERIOD OF STUDY:

May 2018 To December2018 (8 months)

METHODOLOGY:

History was taken on details and duration of fever from a dengue endemic H/o headache , joint pain , nause was noted . Platelet count, prothrombin time and INR,liver function tests including serum bilirubin, serum transaminases, serum albumin was estimated.

LABORATORY INVESTIGATIONS:

Platelet count,liver function tests including serum bilirubin, albumin,globulin,transaminases, prothrombin time activated partial thromboplastin time and INR.

COLLABORATING DEPARTMENTS:

Department Of Medical Gastroenterology

Department Of Biochemistry

Department of Radiology

ETHICAL CLEARANCE: Clearance obtained

CONSENT: Individual written and informed consent obtained.

STATISTICAL ANALYSIS:

All data were entered in Excel 2007 and statistical analysis was performed using the statistical software SPSS 16.0.Data were expressed as frequency (with percentages), median values (with range (min, max)). For continuous variables, Mann Whitney U-test was performed to find the differences between two groups and for categorical variables Pearson's chi-square test was performed.Results were defined as statistically significant when the *P* value (2-sided) was less than 0.05

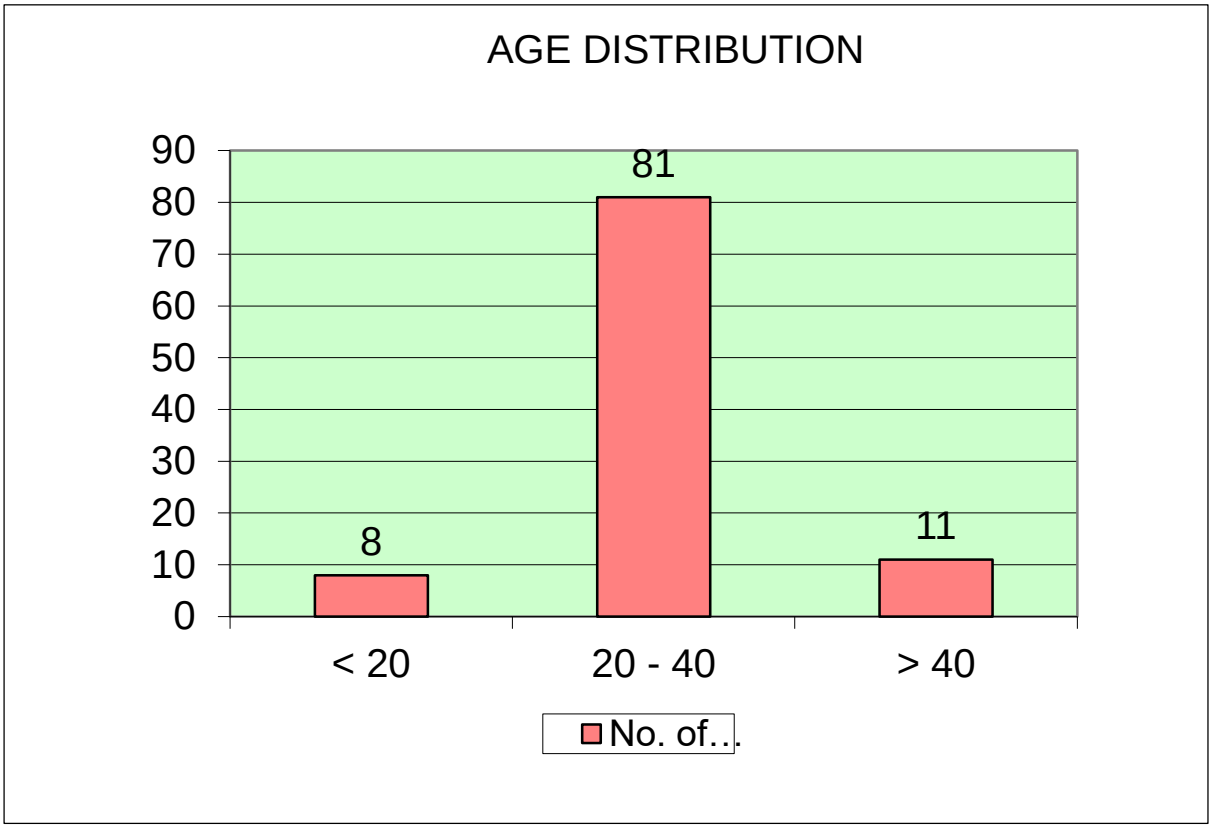
CONFLICT OF INTEREST: NIL

FINANCIAL SUPPORT: SELF

RESULTS AND OBSERVATIONS

1) Age distribution :

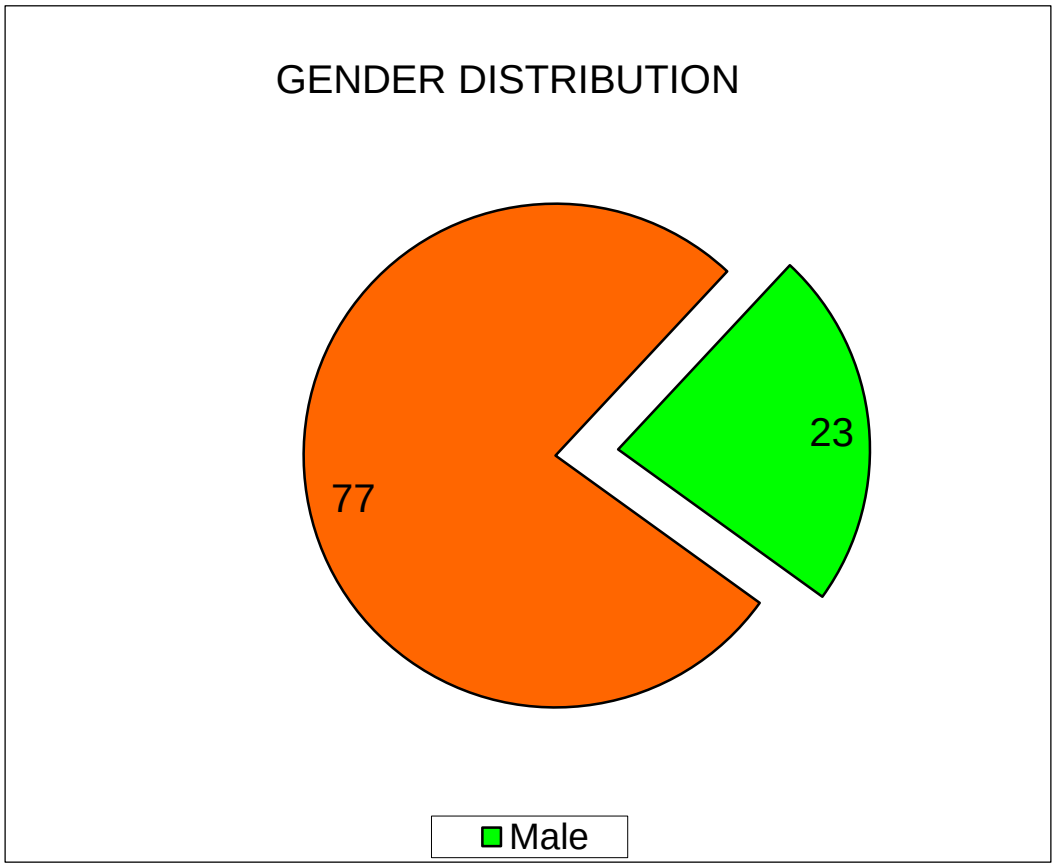
AGE	No. of cases
< 20	8
20 – 40	81
> 40	11
Total	100



Comments : dengue is prevalent among middle age group individuals

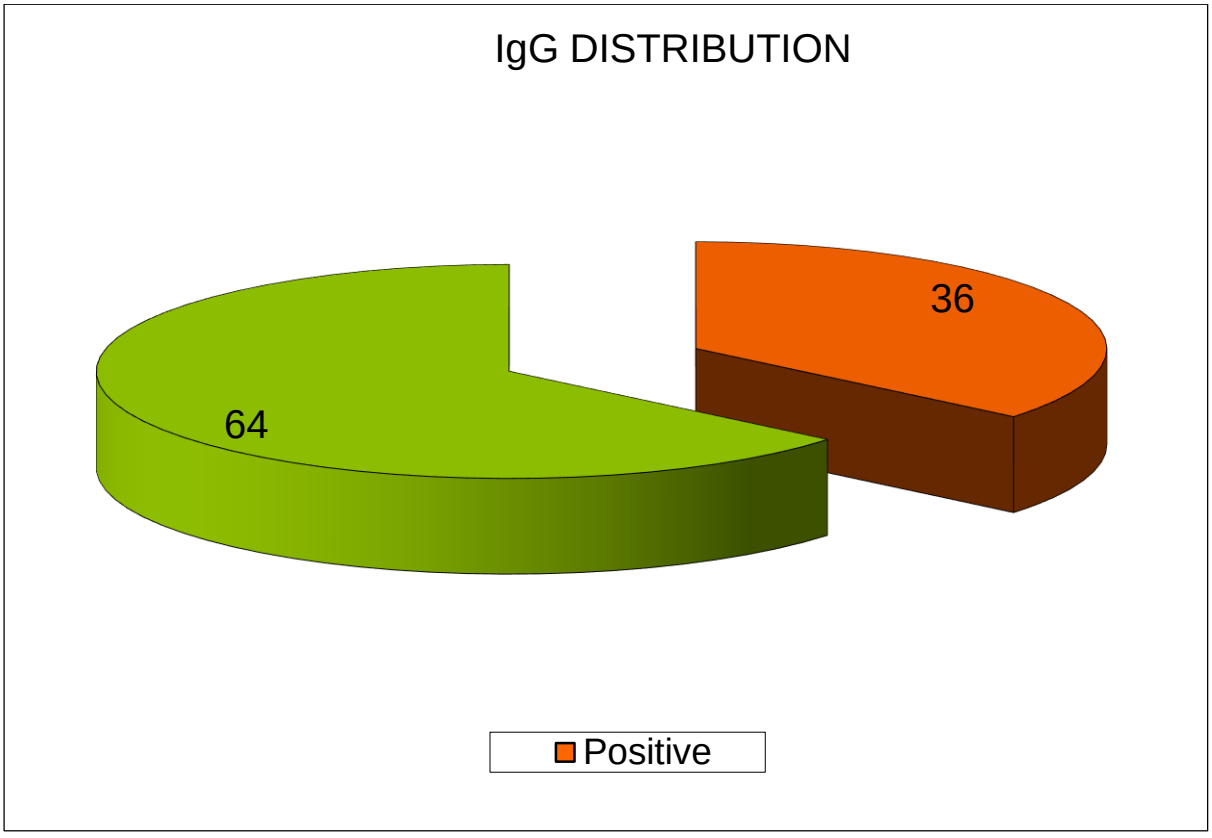
2) Gender distribution:

GENDER	No. of cases
Male	23
Female	77
Total	100



3) Percentage of secondary dengue :

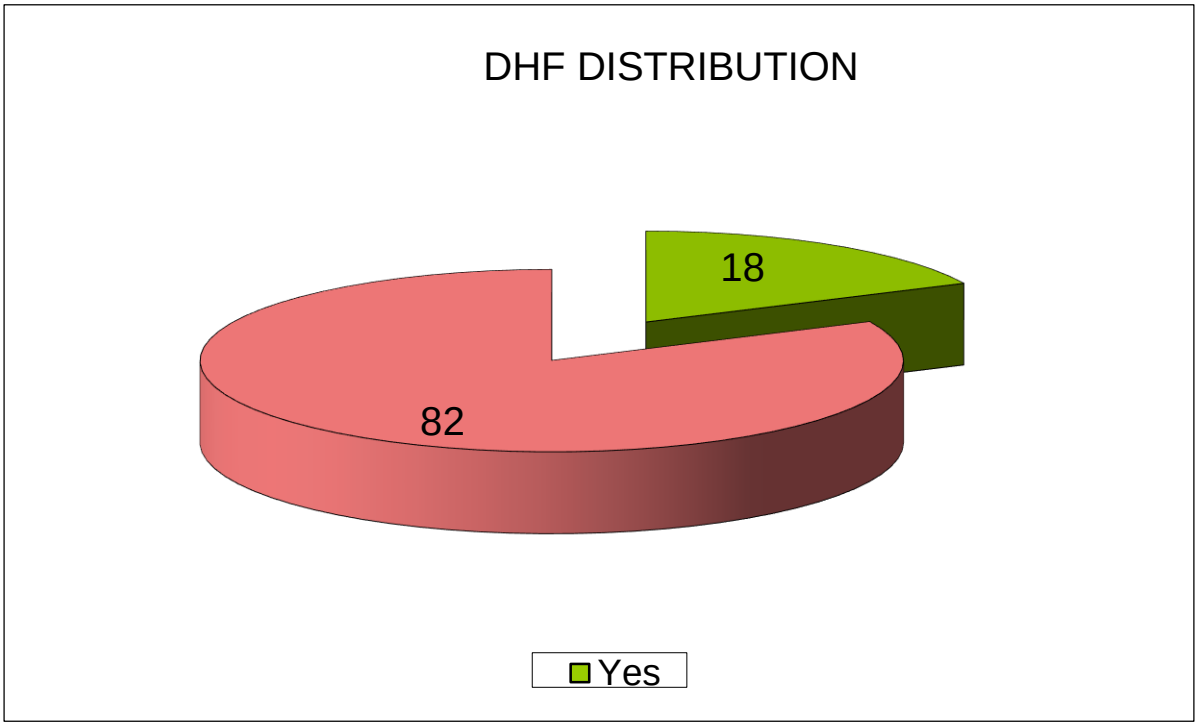
IGG	No. of cases
Positive	36
Negative	64
Total	100



Comments: out of 100 Dengue patients 36 were of secondary dengue

4) Dengue haemorrhagic fever incidence:

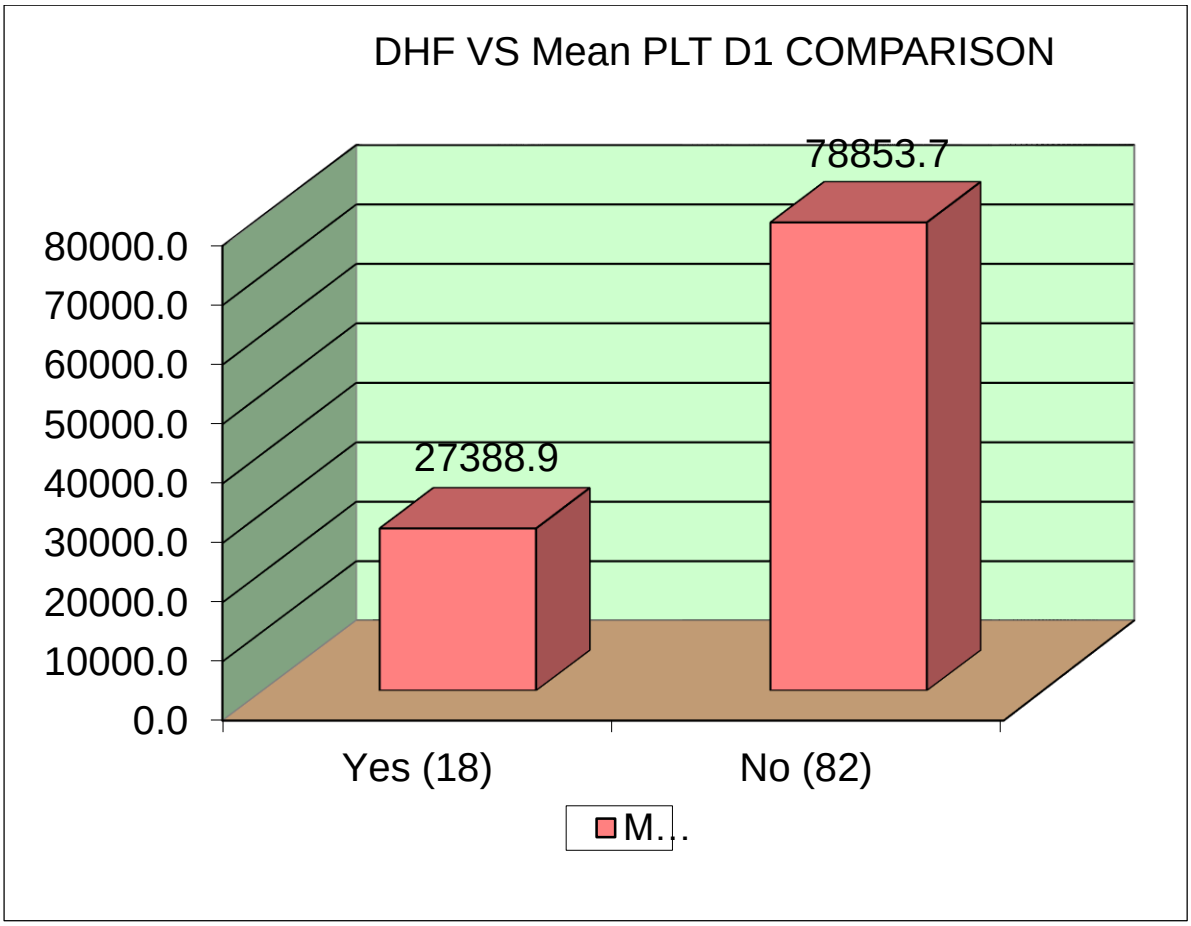
DHF	No. of cases
Yes	18
No	82
Total	100



Comments: out of 100 Dengue patients 18 went to develop Dengue Haemorrhagic fever .

5) Mean platelet on day 1 in DHF vs dengue:

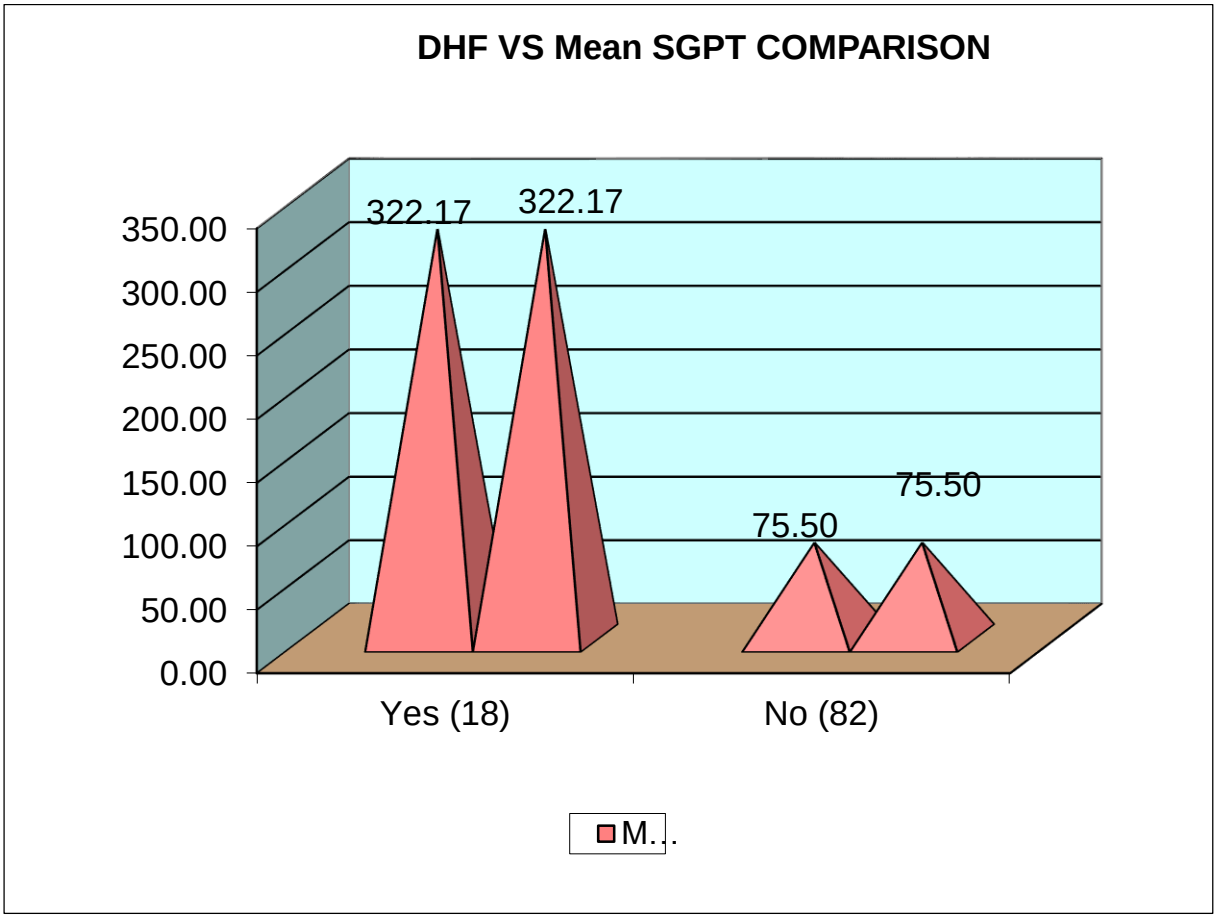
DHF vs PLT D1	Mean	S.D	p' value	
Yes (18)	27388.9	6954.8		
No (82)	78853.7	12639.5	<0.001	Significant



Comments : The patients in the DHF side had lower platelet counts when compared to the Dengue fever wing on Day 1 .

6) SGPT in DHF vs Dengue fever

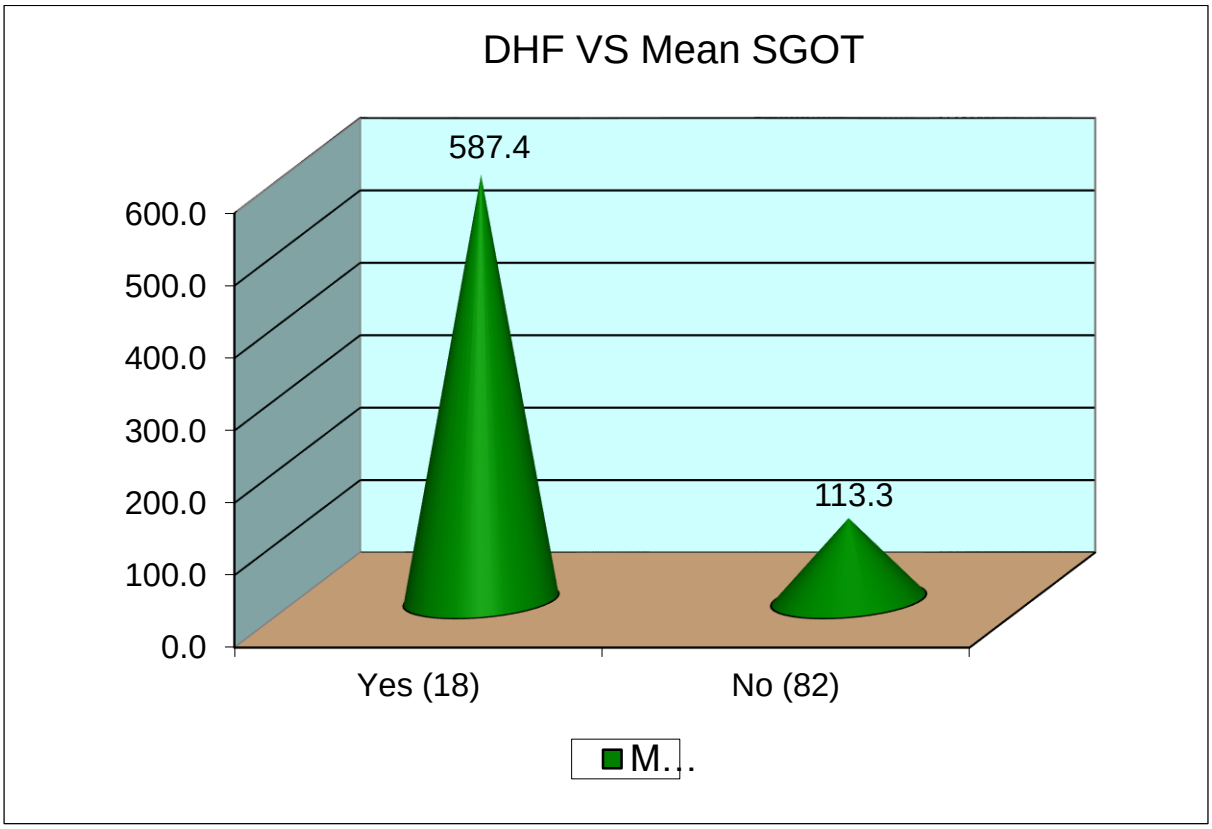
DHF vs SGPT	Mean	S.D	p' value	
Yes (18)	322.17	180.71		
No (82)	75.50	86.90	<0.001	Significant



Comments : There is statistically significant elevation of SGPT in DHF

7) SGOT in DHF vs Dengue fever:

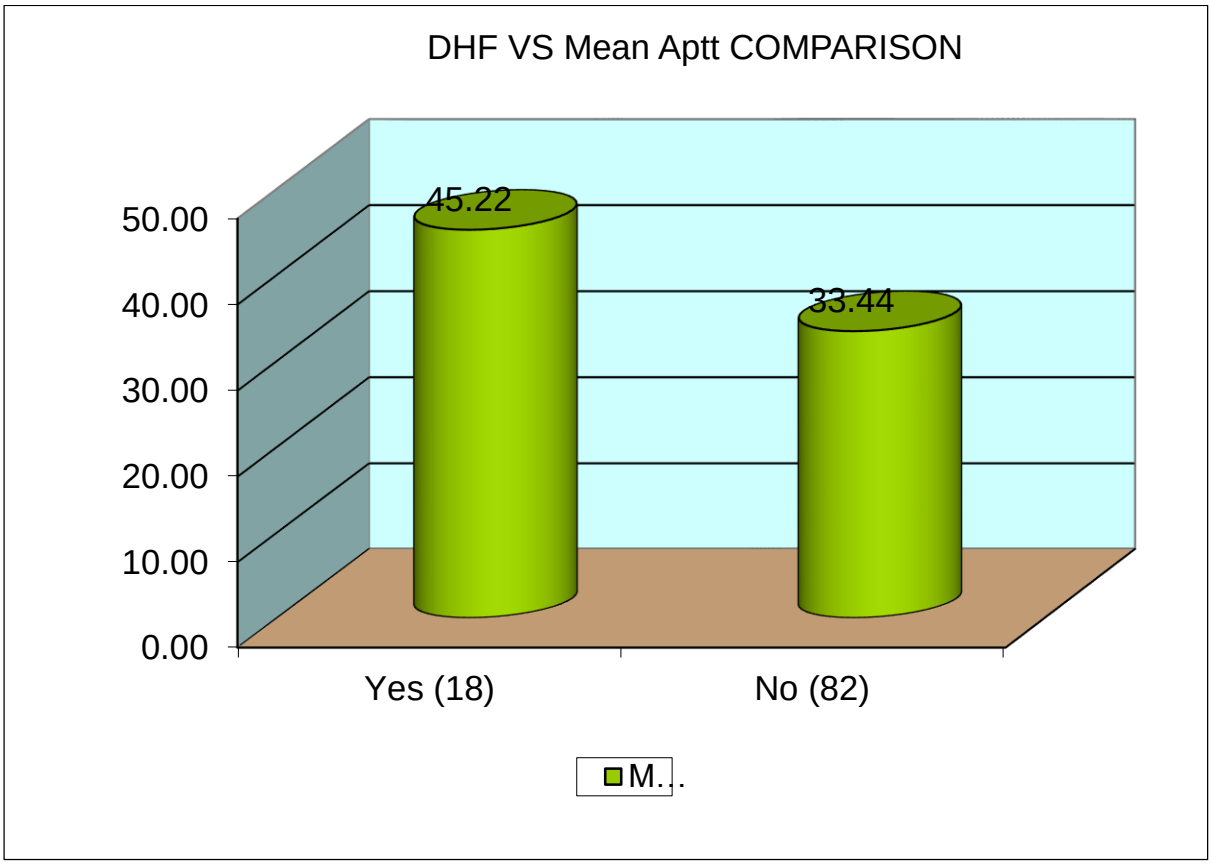
DHF vs SGOT	Mean	S.D	p' value	
Yes (18)	587.4	276.9		
No (82)	113.3	108.8	<0.001	Significant



Comments : There is significant elevation of SGOT in DHF patients . there is significant elevation of SGOT when compared to SGPT

9) aPTT in DHF vs Dengue fever:

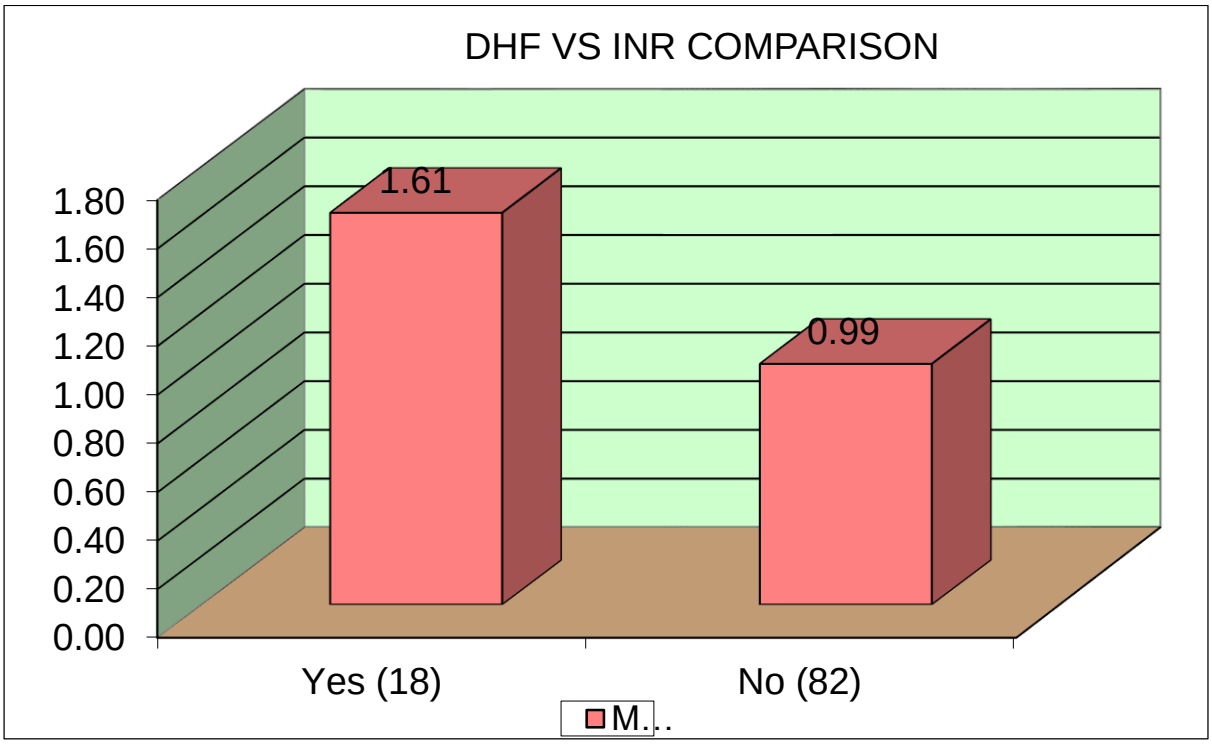
DHF vs Aptt	Mean	S.D	p' value	
Yes (18)	45.22	7.08		
No (82)	33.44	3.30	<0.001	Significant



Comments : Statistically significant elevation of aPTT among DHF patients when compared to dengue fever patients

10) INR in DHF vs Dengue :

DHF vs INR	Mean	S.D	p' value	
Yes (18)	1.61	0.39		
No (82)	0.99	0.22	<0.001	Significant



Comments : INR elevation also corresponds to the severity of dengue but doesn't correlate with the aPTT values hence can not be statistically significant

DISCUSSION

- Our study was done to assess the usefulness of aPTT in predictin th severity of dengue in a tertiary hospital in Madurai
- Among the dengue patients there were 81 patients in 20 to 40 age group 11 patients were more than 40 , 8 patients were less than 20 years of age
- Among the gender predilection , females were affected more than males
- Secondary dengue was found to involve about 36 patients 36 percent had IgG positivity
- Among the dengue fever patients 18 patients had dengue haemorrhagic fever and developed complications and were needing treatment
- The mean platelet count among dengue fever patients were 76,300

  And the mean platelet count among the patients with dengue haemorrhage fever was 27,000

  The average platelet count fall occurred during the 4^{th} day and was more severe among the dengue haemorrhagic fever patients
- The average SGOT in dengue haemorrhagic fever patients was 587 and among dengue fever patietns was 113 . there was statistically

significant elevation . dengue haemorrhagic fever patients had a higher SGOT whn compared to the Dengue fever patients

- The average SGPT in dengue haemorrhagic fever patients was 322 and among dengue fever patietns was 75 . there was statistically significant elevation . dengue haemorrhagic fever patients had a higher SGPT whn compared to the Dengue fever patients but not as statisticall significan as SGOT
- The average PT in dengue haemorrhagic fever patients was 17.8 and among dengue fever patietns was 12.4 . there was statistically significant elevation . dengue haemorrhagic fever patients had a higher prothrombin time whn compared to the Dengue fever patients
- The average INR in dengue haemorrhagic fever patients was 1.61 and among dengue fever patietns was 0.99. there was statistically significant elevation . dengue haemorrhagic fever patients had a higher SGOT whn compared to the Dengue fever patients
- The average aPTT in dengue haemorrhagic fever patients was 45.22 and among dengue fever patietns was 33.2 . there was statistically significant elevation . dengue haemorrhagic fever patients had a higher aPTT whn compared to the Dengue fever patients

SUMMARY

Dengue is a dangerous infectious disease threatening the general population and the particularly viable population . the most feared complications of dengue are due to the secondary effusions and the fluid leakage . The most dreaded complication Dengue Haemorrhagic fever which is easily detectable by the ominous presence of fluid leakage and fall in platelet count and elea=vation in PCV due to hemoconcentration . There is need for firther easy bed side tests to detect the development of Dengue Haemorrhagic fever among Dengue patients . Our study focused on the involvement of liver enzymes and aPTT in dengue haemorrhagic fever . There is a statistically significant elevation of SGOT and SGPT among patients with DHF , More than that there is a significant elevation of aPTT among patients with Dengue Haremorrhagic fever . This can be utilized as a good tool in detecting the patients who might land up in dengue Haemorrhagic fever. This can be anticipated and adequate fluid resuscitation and timely initiation of platelet transfusion prevent serious adverse outcomes .

Thus, this study emphasizes on the role of coagulation in the pathogenesis and prognostication in severe dengue The APTT prolongation occurs even before immunological changes starts. It can predict patients likely to develop complications. This simple cheaper blood investigation of other coagulation function PT, INR, and liver enzymes, can be used as a supporting evidence for severe dengue and thus helps in early intervention and management which can reduce the mortality of dengue fever.

CONCLUSION

1) SGOT and SGPT elevation proved to be an early predictor in the progression of Dengue fever to Dengue Haemorrhagic fever

2) aPTT prolongation proved to be an even early predictor in the progression of Dengue to Dengue haemorrhagic fever and hence rapid intervention prevented the dreaded complications of dengue .

BIBLIOGRAPHY

1. World Health Organization. Global Strategy for Dengue Prevention and Control: WHO 2012-2020
2. Limkittikul K, Brett J, L'Azou M. Epidemiological trends of dengue disease in Thailand (2000-2011): a systematic literature review. PLoS Negl Trop Dis. 2014;8(11):e3241.
3. Isarangkura PB, Pongpanich B, Pintadit P, Phanichyakarn P, Valyasevi A. Hemostatic derangement in dengue haemorrhagic fever. Southeast Asian J Trop Med Public Health. 1987;18(3):331-
4. Van Gorp EC, Setiati TE, Mairuhu AT, Suharti C, Cate Ht, Dolmans WM et al. Impaired fibrinolysis in the pathogenesis ofdengue hemorrhagic fever. J Med Virol. 2002 Aug; 67(4):549-54.
5. Roy A, Sarkar D, Chakraborty S, Chaudhuri J, Ghosh P. Profile of hepatic involvement by dengue virus in dengue infected children. N Am J Med Sci. 2013 Aug;5(8):480-5.
6. Lin SW, Chuang YC, Lin YS, Lei HY, Liu HS, Yeh TM. Dengue virus nonstructural protein NS1 binds to prothrombin/thrombin and inhibits prothrombin activation. J Infect. 2012;64:325-34.
7. Cabello-Gutiérrez C, Manjarrez-Zavala ME, Huerta-Zepeda A, Cime-Castillo J, Monroy-Martínez V, Correa BB et al. Modification of the cytoprotective protein C pathway during Dengue virus infection of

human endothelial vascular cells. Thromb Haemost. 2009 May;101(5):916-28.

8. Sosothikul D, Seksarn P, Pongsewalak S, Thisyakorn U, Lusher J. Activation of endothelial cells, coagulation and fibrinolysis in children with Dengue virus infection. Thromb Haemost. 2007 Apr;97(4):627-34.
9. Orsi FA, Angerami RN, Mazetto BM, Quaino SK, Santiago-Bassora F, Castro V, et al. Reduced thrombin formation and excessive fibrinolysis are associated with bleeding complications in patients with dengue fever: a case-control study comparing dengue fever patients with and without bleeding manifestations. BMC Infect Dis. 2013 Jul 28;13:350.
10. Huerta-Zepeda A, Cabello-Gutiérrez C, Cime-Castillo J, Monroy-MartínezV, Manjarrez-Zavala ME, Gutiérrez-Rodríguez M et al. Crosstalk between coagulation and inflammation during Dengue virus infection. Thromb Haemost. 2008 May;99(5):936-43.
11. Chen LC, Shyu HW, Lin HM, Lei HY, Lin YS, Liu HS et al. Dengue virus induces thrombomodulin expression in human endothelial cells and monocytes in vitro. J Infect. 2009 May;58(5):368-74.
12. Budastra N, Arhana BNP, Mudita IB. Plasma prothrombin time and activated partial thromboplastin time as predictors of bleeding manifestations during dengue haemorrhagic fever. Paediatrica Indonesiana 2009; 49(2): 69-74.

13. Huang Y, Liu C, Wang S, Lei H, Liu H, Lin Y, et al. Activation of Coagulation and Fibrinolysis during Dengue Virus Infection. Journal of Medical Virology 2001; 63(3):247-51. http://dx.doi.org/10.1002/10969071(200103) 63:3<247::AID-JMV1008>3.0.CO;2-F

14. Chuansumrit A, Tangnararatchakit K. Pathophysiology and management of dengue hemorrhagic fever. Transfusion Alternatives in Transfusion Medicine. 2006; 8(suppl s1):3-11. http://dx.doi.org/10.1111/j.1778428X.2006. 00025.x

15. Liu J, Khor B, Lee C, Lee I, Chen R, Yang KD. Dengue haemorrhagic fever in Taiwan. Dengue Bulletin. 2003; 27:19-24.17. Al-Busafi SA, Ghali P, Wong P, Callaway E. Dengue fever climbs the social ladder. *Nature* 2007*;* 448: 734-735.

16. Munasinghe DR, Amarasekera PJ and Fernando CF. An epidemic of dengue-like fever in Ceylon (chikungunya--a clinical and haematological study. *Ceylon Medical Journal* 1966; 11: 129-42.

17. Mendis NM. Epidemiology of dengue-like fever in Ceylon. *Ceylon Medical Journal*

1967; 12: 67-74.

18. Gagnon SJ, Mori M, Kurane I, et al. Cytokine gene expression and protein production in peripheral blood mononuclear cells of children

with acute dengue virus infections. *Journal of Medical Virology* 2002; 67: 41-6.

19. Atukorale V, Meedin, F., Malavige, G.N., Wijesinghe, T, Jayaratne, S.D., Fernando, N., Ogg, G.S.,. Molecular characteristics of dengue viral serotypes responsible for dengue epidemic in year 2010.In: *Sri Lanka Medical Association Annual Congress. Colombo, 2011*

20. Kanakaratne N, Wahala WM, Messer WB, et al. Severe dengue epidemics in Sri Lanka, 2003-2006. *Emerging Infectious Diseases* 2009; 15: 192-9.

21. Weaver SC, Vasilakis N. Molecular evolution of dengue viruses: contributions of phylogenetics to understanding the history and epidemiology of the preeminent arboviral disease. *Infection Genetics and Evolution* 2009; 9: 523-40.

22. Mathew A, Rothman AL. Understanding the contribution of cellular immunity to dengue disease pathogenesis. *Immunological Reviews* 2008; 225: 300-13.

23. Guzman MG, Kouri G, Valdes L, Bravo J, Vazquez S and Halstead SB. Enhanced severity of secondary dengue-2 infections: death rates in 1981 and 1997 Cuban outbreaks. *Revista Panamericana de Salud Pública*2002*;* 11: 223-7.

24. Messer WB, Gubler DJ, Harris E, Sivananthan K and de Silva A M. Emergence and global spread of a dengue serotype 3, subtype III virus. *Emerging Infectious Diseases* 2003; 9: 800-9.

25. Kyle JL, Harris E. Global spread and persistence of dengue. *Annual Review of Microbiology* 2008; 62: 71-92.

26. Duyen HT, Ngoc TV, Ha DT, et al. Kinetics of Plasma Viremia and Soluble Nonstructural Protein 1 Concentrations in Dengue: Differential Effects According to Serotype and Immune Status. *Journal of Infectious Diseases* 2011; 203:1292- 300.

27. Nguyen TP, Kikuchi M, Vu TQ, et al. Protective and enhancing HLA alleles, HLA- DRB1*0901 and HLA-A*24, for severe forms of dengue virus infection, dengue hemorrhagic fever and dengue shock syndrome. *PLoS Neglected Tropical Diseases* 2008; 2: e304.

28. Appanna R, Ponnampalavanar S, Lum Chai See L and Sekaran SD. Susceptible and protective HLA class 1 alleles against dengue fever and dengue hemorrhagic fever patients in a Malaysian population. *PLoS One* 2010; 5: e13029.

29 . Chau TN, Quyen NT, Thuy TT, et al. Dengue in Vietnamese infants--results of infection-enhancement assays correlate with age-related disease epidemiology, and cellular immune responses correlate with disease severity. *Journal of Infectious Diseases* 2008; 198: 516-24.

30 Thomas L, Verlaeten O, Cabie A, et al. Influence of the dengue serotype, previous dengue infection, and plasma viral load on clinical presentation and outcome during a dengue-2 and dengue-4 co-epidemic. *American Journal of Tropical Medicine & Hygiene* 2008; 78: 990-8.

31 Wang WK, Chao DY, Kao CL, et al. High levels of plasma dengue viral load during defervescence in patients with dengue hemorrhagic fever: implications for pathogenesis. *Virology* 2003; 305: 330-8.

32 Guilarde AO, Turchi MD, Siqueira JB, Jr., et al. Dengue and dengue hemorrhagic fever among adults: clinical outcomes related to viremia, serotypes, and antibody response. *Journal of Infectious Diseases* 2008; 197: 817-24.

33 Mathew A, Kurane I, Green S, et al. Predominance of HLA-restricted cytotoxic T- lymphocyte responses to serotype-cross-reactive epitopes on nonstructural proteins following natural secondary dengue virus infection. *Journal of Virology 1998;* 72: 3999-4004.

34 Beaumier CM, Mathew A, Bashyam HS and Rothman AL. Cross-reactive memory CD8(+) T cells alter the immune response to heterologous secondary dengue virus infections in mice in a sequence-specific manner. *Journal of Infectious Diseases* 2008; 197: 608-17.

35 Simmons CP, Dong T, Chau NV, et al. Early T-cell responses to dengue virus epitopes in Vietnamese adults with secondary dengue virus infections. *Journal of Virology* 2005; 79: 5665-75.

36 Durbin AP, Vargas MJ, Wanionek K, et al. Phenotyping of peripheral blood mononuclear cells during acute dengue illness demonstrates infection and increased activation of monocytes in severe cases compared to classic dengue fever. *Virology* 2008; 376: 429-35.

37 Dong T, Moran E, Vinh Chau N, et al. High pro-inflammatory cytokine secretion and loss of high avidity cross-reactive cytotoxic T-cells during the course of secondary dengue virus infection. *PLoS One* 2007; 2:e1192.

38 World Health Organization and Tropical Diseases Research. Dengue: Guidelines for diagnosis,treatment, prevention and control. Geneva: World Health Organization; 2009: new edition.

39 World Health Organization. First report on neglected tropical diseases: working to overcome the globalimpact of neglected tropical diseases. Geneva: World Health Organization; 2010.

40 World Health Organization. Global strategy for dengue prevention and control – 2012–2020. Geneva: World Health Organization; 2012..

41 Suaya JA, Shepard DS, Siqueira JB, Martelli CT, Lum LCS, Tan LH, et al. Cost of dengue cases in eight countries in the Americas and Asia:

a prospective study. American Journal of Tropical Medicine and Hygiene. 2009;80:846–855.

42 World Health Organization. Comprehensive guidelines for prevention and control of dengue and dengue hemorrhagic fever. New Delhi: WHO, SEARO; 2011: revised and expanded edition.

43 Baruah K, Dhariwal AC. Epidemiology of dengue, its prevention and control in India. Journal of Indian Medical Association. 2011;109 (2):82–6.

44 Baruah K, Biswas A, Suneesh K, Dhariwal AC. Dengue fever: Epidemiology and clinical pathogenesis

45 Dutta AK, Biswas A, Baruah K, Dhariwal AC. National guidelines for diagnosis and management of dengue fever/dengue hemorrhagic fever and dengue shock syndrome. J Ind Med Assn. 2011;109

46 Dash AP, Bhatia R, Kalra NL. Dengue in South East Asia: An appraisal of case management and vector control. Dengue Bulletin. 2012;36:1–12.

47 Chen L, Wilson ME. Non-vector transmission of dengue and other mosquito-borne flaviviruses. Dengue Bull. 2005;29:18–30.

PROFORMA

Name:

Age / Sex:

IP no:

Occupation:

Presenting complaints:

h/o,fever , joint pain , myalgia , bleeding diathesis , diarrhoea , retroorbital pain.

Past History:

h/o bleeding diathesis , liver disease , kidney disease ,

h/o previous infection with dengue

h/o,HEPATITIS.

Personal history

alcoholic/ non alcoholic

smoker/ nonsmoker

Clinical Examination:

General Examination:

Consciousness, orientation, febrile/afebrile, Pallor, jaundice, Clubbing,

Lymphadenopathy, pedal edema.

Vitals:

PR

BP

RR

SpO2

Systemic examination:

CVS:

RS:

ABDOMEN:

CNS:

Laboratory investigations:

1.platelet count

2.LFTs including serum bilirubin,albumin,globulin,transaminases,

3. prothrombin time aPtt and INR.

Diagnosis

LIST OF ABBREVIATION

LFT	-	liver function test
AST	-	Aspartate aminotransferase
ALT	-	Alanine aminotransferase
PT	-	prothrombin time
aptt	-	Activated partial thromboplastin time

MASTER CHART

S NO	Name	I.P No.	AGE	SEX	IgG	DHF	PLT D1	PLT D4	SGOT	SGPT	PT	Aptt	INR
1			34	M	N	N	98000	65000	112	98	12	34	0.8
2			23	M	P	N	74000	70000	134	99	14	33	0.9
3			34	M	N	N	65000	45000	223	105	13	36	1.5
4			34	F	P	N	69000	55000	113	89	14	31	1
5			26	M	N	N	78000	59000	145	88	12	29	1.2
6			45	M	N	N	68000	63000	65	54	11	30	1
7			26	M	N	N	88000	60000	23	21	14	32	0.8
8			23	M	P	N	87000	58000	99	60	18	39	1.7
9			43	M	N	N	65000	45000	154	114	12	36	1
10			55	F	N	N	88000	63000	116	102	11	34	0.8
11			33	M	N	N	68000	60000	123	110	12	35	1
12			23	F	P	N	88000	59000	66	50	14	34	1.2
13			23	M	N	N	65000	45000	95	60	12	36	0.9
14			34	F	N	N	88000	63000	63	45	11	35	0.8
15			40	M	P	Y	30000	28000	466	255	20	48	1.8
16			22	M	N	N	68000	63000	66	40	14	36	1
17			21	M	N	N	65000	45000	95	50	12	34	1.5
18			22	M	P	N	68000	60000	98	58	14	36	0.8
19			32	M	N	N	98000	65000	63	62	12	29	0.9
20			29	F	N	Y	33000	36000	595	420	18	43	1.9
21			16	M	P	N	74000	65000	45	40	14	29	0.8
22			50	M	N	N	98000	65000	55	59	12	34	1
23			25	F	P	N	68000	63000	66	20	14	36	0.9
24			23	M	N	N	65000	45000	62	24	12	29	1.2
25			36	M	P	Y	27000	22000	390	265	17	44	1.8
26			32	F	N	N	74000	48000	63	45	14	29	0.8
27			33	M	N	N	98000	58000	95	80	12	34	1
28			34	M	N	N	68000	60000	89	65	11	35	1.3
29			23	M	N	N	65000	45000	88	66	12	34	0.9
30			24	M	P	Y	28000	22000	677	440	12	28	1
31			25	M	N	N	68000	60000	84	56	14	29	0.8
32			22	F	N	N	98000	58000	442	325	12	34	0.8
33			32	M	N	N	65000	63000	81	65	14	29	0.9
34			29	M	P	Y	40000	35000	334	225	20	62	1.9
35			26	M	N	N	68000	63000	88	50	14	29	0.9
36			23	F	N	N	98000	58000	89	54	14	34	1
37			39	M	P	Y	21000	19000	789	455	19	43	2
38			45	M	P	N	74000	65000	63	48	14	29	1
39			55	M	N	N	68000	63000	116	90	12	29	0.9
40			56	F	N	Y	38000	33000	298	115	20	44	1.6
41			29	M	P	N	68000	63000	24	10	11	35	1
42			30	M	N	N	98000	58000	90	56	12	29	1.2
43			31	M	P	Y	22000	19000	887	560	19	54	1.9
44			33	M	N	N	65000	45000	98	65	14	36	0.9
45			27	F	N	N	98000	65000	46	32	14	29	1
46			28	M	N	Y	35000	20000	289	115	18	49	1.8
47			29	M	P	N	98000	45000	56	25	12	36	1
48			31	M	N	N	74000	63000	55	26	14	29	0.8
49			20	M	P	Y	22000	20000	339	105	13	44	0.8
50			28	F	P	N	68000	63000	98	23	14	29	1

50			28	F	P	N	68000	63000	98	23	14	29	1
51			29	M	N	N	98000	59000	49	65	12	36	1
52			30	M	P	Y	19000	16000	567	422	19	47	1.6
53			31	M	N	N	65000	45000	16	14	13	29	1
54			35	M	P	N	68000	63000	22	28	12	36	1
55			26	F	N	Y	28000	19000	980	225	14	41	0.8
56			29	M	P	N	88000	63000	33	13	13	36	0.8
57			27	M	N	N	65000	45000	98	23	12	36	0.9
58			23	M	P	Y	27000	19000	347	115	17	43	2.1
59			23	M	N	N	88000	63000	45	25	14	36	0.9
60			22	F	N	N	88000	45000	599	434	14	36	0.8
61			29	M	P	N	74000	65000	32	15	12	30	1
62			56	M	N	N	88000	45000	89	56	15	35	0.8
63			54	M	N	N	88000	63000	145	65	12	30	0.8
64			45	M	P	Y	17000	16000	990	709	19	39	1.9
65			44	F	P	N	65000	45000	225	98	14	30	1
66			31	M	N	N	74000	54000	20	10	12	35	1
67			26	M	N	N	88000	63000	99	65	14	39	1.9
68			23	M	N	N	65000	45000	205	105	13	35	1
69			22	F	P	N	98000	65000	125	118	14	36	0.8
70			29	M	N	N	74000	45000	115	105	12	35	1
71			30	M	N	N	88000	63000	145	95	19	42	1.6
72			31	M	N	N	98000	65000	165	65	11	35	1
73			35	F	N	N	88000	54000	98	62	14	34	0.8
74			36	M	P	N	74000	63000	156	115	11	36	1
75			35	M	N	Y	16000	15000	879	300	20	44	1.4
76			32	M	N	N	65000	45000	125	95	11	30	1
77			30	M	N	N	88000	63000	198	99	14	30	0.8
78			22	M	N	N	65000	45000	600	500	12	36	0.9
79			29	F	N	N	74000	63000	42	24	13	41	1.3
80			26	M	P	N	67000	55000	65	45	12	29	1
81			24	M	N	N	74000	45000	35	25	13	35	0.8
82			28	F	P	Y	27000	20000	356	275	17	54	1.6
83			23	M	N	N	76000	45000	65	25	14	29	1
84			25	M	P	N	88000	65000	56	22	14	34	0.8
85			25	M	N	N	74000	63000	59	26	13	29	1
86			24	M	N	N	98000	65000	58	23	13	34	0.9
87			29	F	P	N	77000	45000	94	35	13	36	0.8
88			30	M	N	N	88000	63000	49	56	12	35	0.9
89			33	M	N	N	74000	60000	145	65	11	39	1.1
90			31	M	P	N	98000	59000	456	424	14	30	0.8
91			35	F	N	N	88000	60000	126	65	13	34	0.9
92			38	M	P	N	65000	45000	26	15	12	35	1.3
93			19	M	N	N	88000	60000	69	14	13	30	0.9
94			16	F	N	N	74000	56000	113	90	12	34	1.2
95			17	M	P	N	56000	45000	145	98	11	30	0.9
96			13	M	N	N	98000	65000	45	14	13	36	0.8
97			13	M	N	Y	34000	28000	1067	600	21	46	1.6
98			16	F	P	N	65000	45000	62	25	13	35	0.8
99			19	M	P	Y	29000	23000	324	198	18	41	1.5
100			25	M	N	N	98000	65000	334	201	16	39	1.4

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Course	:	PG in MD., General Medicine
Period of Study	:	2016-2019
College	:	MADURAI MEDICAL COLLEGE
Research Topic	:	Association of abnormal coagulation profile and liver enzymes in dengue infection and their significance in assessing severity of the disease.
Ethical Committee as on	:	13.04.18

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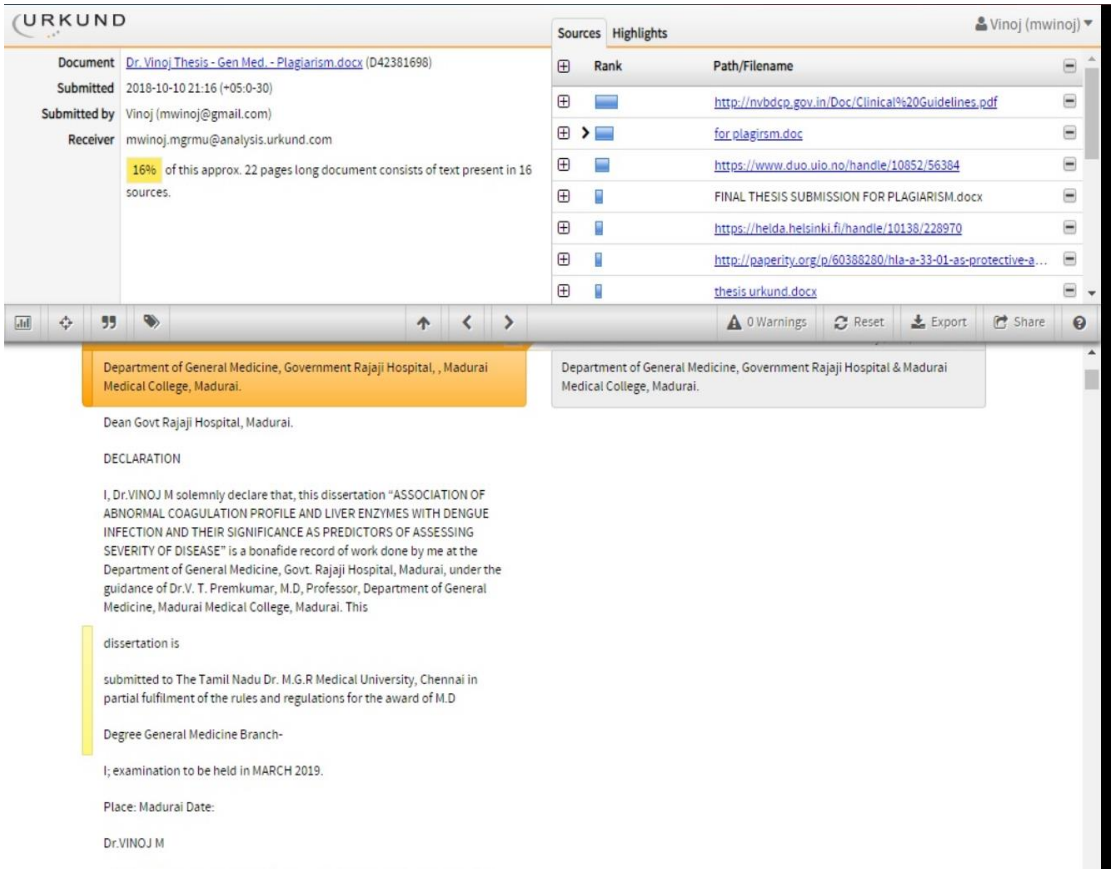
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Place: Madurai Date:
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This is to certify that this dissertation titled **"ASSOCIATION OF ABNORMALCOAGULATION PROFILE AND LIVER ENZYMES WITH DENGUE INFECTION AND THEIR SIGNIFICANCE AS PREDICTORS OF ASSESSING SEVERITY OF DISEASE"** of the candidate **Dr. VINOJ M** for the award of **M.D** degree in the branch of GENERAL MEDICINE. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file containing from introduction to conclusion pages and result shows **1** percentage of plagiarism in the dissertation.

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